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PHYSIOLOGICAL REVIEWS

VOL. III OCTOBER, 1923 No. 4

OESTRUS, OVULATION AND MENSTRUATION

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A long series of traditional and scientific writings testifies to the mystification produced by the menstrual periodicity of the human female. We find the Mosaic lawgivers already concerned to regulate the hygiene of menstruation; thousands of years later an Arrhenius is still puzzling over the relation between the cycles of menstruation and those of the moon. In recent years, however, there seems to have been a genuine advance toward a biological explanation of the reproductive cycle in mammals, and we may hope that the general enlightenment is finally extending to the undoubtedly peculiar problems of our own species. We now know that the discharge of ova from the ovarian follicles is a periodic function, accompanied by alterations in the female organism which provide for protection and nutrition of the embryos in their wandering and development; but the effort to test and amplify this general idea and to give it practical application in the understanding of such special cases as the human menstrual cycle has continually been hampered by a diversity of detail so great that each species seems to present a special problem. Outside the mammalian class we find such extremes as the seventeen-year cycle of the locust, *Cicada septendecim*, and the daily ovulation of the domestic hen; among the mammals we have to reckon with such different phenomena as the spectacular annual rutting of certain deer; the "heat" of horse, cow and pig, recurring perennially at three-weekly intervals; the almost totally inconspicuous oestrus of the laboratory rodents; and the menstrual hemorrhagic process of the higher primates. The dissimilarity of external manifestations is matched by the different structure of the reproductive tract and the multiplicity of form in placentation and in the embryonic membranes. The problem which presents itself to students of these phenomena is to learn the relation of ovulation to the outward events of the cycle, to follow in detail the accompanying changes of form and function in the

reproductive tract, and ultimately, let us hope, to comprehend their physiological interrelations. It is clear that the solution of the cycle of any one species must depend in part upon the progress of knowledge of them all, and that a complicated or obscure cycle like that of the human species will be intelligible only in the light of simpler types. These questions, and the recent attempts to solve them, form the subject of our review.

I. THE REPRODUCTIVE CYCLE IN GENERAL. *Oestrus.* Turning to the simplest type of reproductive manifestations, such as is presented for instance by the ungulates and the carnivores, we observe the recurrence at regular intervals of a condition characterized by sexual excitement and by impregnation of the female if copulation takes place. Between the periods of oestrus,[1] sexual activity is completely in abeyance. The intervals between the recurrences of oestrus, the duration of the period of sexual excitement and the degree of its manifestation are, as we have said, different in various species. The domestic pig, for instance, undergoes a three-day period of oestrus at intervals of about three weeks throughout the year; the sheep has in the autumn or, in some breeds, in spring and autumn a sequence of oestrous periods, which may include three or four cycles unless cut short by impregnation; the dog is in heat for a week or more about twice annually. In cats and dogs the interoestrous interval is longer than the span of gestation, while in the pig and other animals with rapid cycles the reverse is true, and there seems to be a mechanism by which oestrus (and consequently ovulation) is prevented from occurring during pregnancy.

[1] "Oestrus" (heat) is the periodic condition of sexual activity characterized by willingness to mate, as contrasted to the intervals during which animals do not mate. In Heape's terminology (27) the term "prooestrus" indicates the preliminary or premonitory stage immediately preceding oestrus. The interval of quietude following oestrus is called "dioestrus" if it be only a relatively brief interlude between regularly recurrent oestrous periods, "anoestrus" if it be so long as to cover a large part of the year between mating seasons. The sequent periods of prooestrus, oestrus and dioestrus or anoestrus form together the oestrous cycle. As will be seen in this review, we must also consider a post-oestrous stage; and finally, we are now aware that a stage called "metoestrus" by Heape, to indicate a period of recession of reproductive activity immediately after oestrus, does not occur in the original sense except in a few animals which do not ovulate spontaneously. The reader will find in the works of Heape (27) and Marshall (45) a collection of information bearing upon this subject; the recent monograph of Long and Evans (41) contains a tabular statement of the extant data, brought up to date.

Oestrus is not in all species marked by obvious outward signs of sexual excitement. In the rodents especially it has been difficult to establish the existence of regular cycles. In the guinea pig, for example, oestrus is so inconspicuous as often to escape observation even by experienced breeders. Recently, however, it has been discovered by Stockard and Papanicolaou that the ovulation cycle is accompanied by changes in the vaginal secretion, so that by microscopic examination of vaginal smears it is possible in this species to detect a regular cycle of about sixteen days' duration. By this means we have already learned to follow the oestrous cycle of four rodent species hitherto unknown or not fully understood. (Guinea-pig, Stockard and Papanicolaou (74); rat, Long and Evans (41); mouse, Allen (1); rabbit, unpublished observations of Pelkan, quoted by Long and Evans.)

It is hardly necessary to point out the further perplexing dissimilarity between these forms of the oestrous cycle and the reproductive phenomena of the human species. Here neither sexual desire nor the act of mating are limited to special periods, but may occur at any stage of the cycle. The only distinct external event is the appearance of a menstrual hemorrhage, which in spite of its dissociation from a specific oestrus is yet so constant in its recurrence that it inevitably suggests the oestrous periodicity of other mammals.

Ovulation. The onset of oestrus is preceded or accompanied by changes in the ovary, particularly in the Graafian follieles and their enclosed ova. Among the large follicles in a resting stage which are always present in the sexually mature ovary, one follicle (or a group, according to the species) begins to grow still larger and passes during a few days into the fully mature stage. In the pig, for instance, the mature follicles increase from a diameter of 4 or 5 mm. to 8 or 10 mm. During this growth there is a striking development of epithelioid structure in the formerly spindle-shaped cells of the theca interna folliculi. The cumulus öophorus is partially dissociated by thinning and spreading apart of its component granulosa cells so that the ovum is left barely anchored to the unchanged granulosa cells of the follicular wall below it. The ovum itself undergoes the phenomena of maturation, i.e., extrusion of the first polar body and the prophase and metaphase of the second polar division. The result is a distinctive modification of the follicle which is typical of the prooestrous or oestrous periods. The details of these changes in the follicle have long been known to embryologists, but their exact time-relations have only recently been made out in a few species (among them the ferret, Robinson (61); pig, Corner (10), (11);

Seckinger (68); rat, Long and Evans (41)). There has been some inexactness in the literature due to failure to recognize these morphological signs, which are essential to the identification of mature follicles.

After two centuries of discussion which is now chiefly of antiquarian interest it has been established that in most mammals the ripe follicles rupture spontaneously at some time during the oestrus, but it is also known that in three animals the follicles usually do not rupture unless coition occurs, although they undergo the preliminary maturation as in other forms; if mating does not occur, the follicles and their contained ova degenerate. This group includes the domestic cat, the ferret (*Mustelus putorius*) and the domestic rabbit. The significance and mechanism of this specialized type of follicular behavior are at present beyond conjecture. Other animals formerly supposed not to ovulate spontaneously have been removed from this class by more accurate observation.

Rupture of the follicles is affected by some unknown cause. Winiwarter and Sainmont (79) and Guttmacher and Guttmacher (22) suggest the possible action of involuntary muscle fibers in the theca externa, of which the latter authors have presented a full anatomical and pharmacological study. Other outstanding conjectures are those of Clark (8) that rupture is brought about by an increased engorgement of follicular blood vessels at the time of oestrus, and of Schochet (65) that the follicles develop in their cavities a ferment which erodes their walls.

It has been found with singular unanimity for a number of species that the unfertilized ovum passes through the Fallopian tube to degenerate in the uterine cavity, and that the passage into the uterus requires, regardless of the widely variant length of the tube in the different species, about three days' time. There are only three known exceptions to this rule. In the dog it appears that about a week's time is required for passage of the ova into the uterus. In the rat it is stated by Long and Evans (41) that the unfertilized ova do not pass farther than the last part of the Fallopian tubes; the fertilized ova require only the usual three days to reach the uterus. In the opossum (26) the ova pass more rapidly into the uterus than is the case in Eutherian mammals, reaching the uterine cavity about one day after ovulation.[2]

Implantation. The subsequent fate of the ova when fertilized has become for many species a well-grounded part of our embryological

[2] Sobotta (63) has given a useful review of the literature on this topic, up to 1914.

knowledge. So far as known, fertilization always takes place in the Fallopian tubes, and the fertilized ova proceed, exactly as the unfertilized, into the uterine lumen on the fourth day, by which time segmentation has begun. Thereafter (it is hardly necessary to point out) the events of further development, fixation and implantation of the early embryos differ according to the species. In some mammals, for instance the domestic pig, the embryos become fixed to the endometrium by a diffuse placental apposition so simple that there is merely a loose interlocking of fetal with maternal tissue, and no destruction of the endometrium; when at parturition the chorions are freed, they leave behind an intact maternal epithelium. In other ungulates, as the sheep and cow, there is upon the cotyledonary eminences of the endometrium a more extensive interlocking of tissue, with but little endometrial destruction. In carnivores, the embryos after fixation upon the surface of the endometrium form a placental attachment in which the endometrial epithelium and glands are ultimately destroyed, leaving only the blood vessels. In some of the rodents (i.e., the guinea pig) the ovum burrows under the endometrial surface to form a new implantation cavity, and at the same time the uterus reacts to the embryonic attack by the formation of decidual cells from the endometrial stroma. Nidation of the human embryo involves widespread destruction of the endometrium by the trophoblast, even to the opening of maternal blood vessels into intervillous spaces, and a general conversion of the endometrial stroma into decidual cells. It is not strange, in view of these differences in reaction between uterus and embryo, as found in various mammals, that the part played by the uterus in the reproductive cycle is somewhat different in various species.

The corpus luteum. The cavity formed by the rupture of the Graafian follicles is replaced by the transformation of the follicular walls into a new structure, the corpus luteum. The obviously important character of this tissue, or organ, has given rise to a long discussion as to its histogenesis, which is now definitely closed, or at least turned in a new direction, for we are certain that the bulk of the lutein tissue is produced by enlargement (without multiplication) of the cells of the granulosa of the follicle. Exactly what becomes of the theca interna during formation of the corpus luteum is still uncertain, or perhaps is different in various species; it has been described as becoming converted into the fibroblasts of the young corpus luteum (72) in the mouse; as degenerating in situ more or less completely, early or late in the life of the corpus luteum (marmot) (78); (man) (49); as becoming indistinguishably merged with

the granulosa lutein cells (bat) (77); and as remaining as a special cell-type intermingled with the granulosa lutein cells throughout the life of the corpus luteum (pig) (10).[3]

Within three to seven days after ovulation the corpus luteum has become completely organized into a body which by reason of its epithelioid type of structure, consisting of lipoid-laden cells bordered everywhere by large capillaries, has become recognized (following Born, as quoted by Fraenkel (16) and Prenant (57) as resembling the endocrine glands. This recognition, however, rests merely upon general appearances, since rather extensive cytological and microchemical studies have left us without proof of any constant or specific cellular element or secretion peculiar to this tissue. The cell-inclusions described by Regaud and Policard (59), Van der Stricht (77), Cesa-Bianchi (7), Drips (13) and others, are in the reviewer's opinion not of specific nature; similar lipoidal granulations, of equally problematical significance, occur in other endocrine tissues.

If conception follows ovulation, the corpus luteum of that ovulation persists throughout pregnancy; but if the ovum remains unfertilized, the corpus luteum (in most species) survives a much briefer time. In the pig (11), rabbit (56) and guinea pig (37), degeneration takes place after about fifteen days; in the dog (47) it is said to persist as long as one month. Although the species are few in which the duration of the corpus luteum has been worked out with accuracy, we have enough information to justify two conclusions: first, that in all known species the corpus luteum of a non-fertilized ovulation persists at least as long as the time required by the embryos of the same species to become implanted in the uterine cavity; second, with only two known exceptions (the rat and the mouse) the corpus luteum always begins to retrogress before the occurrence of a new ovulation and the consequent production of a new corpus luteum. In the case of the rat and the mouse it is true that the corpora persist so long that the ovaries ultimately contain many successive crops of undegenerate corpora lutea at one time, but even here it has been shown by an ingenious method of Long and Evans (41), (marking the living corpora with vital dyes) that there are cytological changes, presumably of retrogressive nature, which take place in each set of corpora lutea before the production of a new set.

In former years there was an extensive debate as to the existence and significance of anatomical differences between the corpus luteum of

[3] Readers who wish to pursue the literature of this subject further are referred to the last-cited work of the reviewer (10) for a detailed bibliography.

pregnancy (so-called "corpus luteum verum") and that of an unfertilized ovulation ("c.l. spurium"). Now that we know something of the life span of the corpus luteum, it is clear that there is but one type, which however may undergo either of two fates,—retrogression or persistence,—according to the fate of the ovum. A corpus which persists during several months of pregnancy will naturally differ in cytological detail from the younger stages of its existence. Thus the present writer (9) has been able to recognize a series of changes in the lipoid content of the lutein cells of the swine by which stages of pregnancy may be distinguished, but which (excepting the earliest changes) are not developed in the corpora lutea of unfertilized ovulation. J. W. Miller (53) has also described differences in lipoids and in calcium-content in the two varieties of the human corpus luteum. In swine, and apparently also in man, the corpora lutea continue to grow in size for a time after implantation of the embryo, and hence they reach a slightly larger diameter than those which grow but two weeks: and in swine at least they develop a heavier framework of connective tissue. It is therefore possible for an experienced observer to determine by microscopic study of a sow's corpora lutea whether or not there is a pregnancy of the second month or later, but the corpora lutea of earlier stages are not distinguishable as to pregnancy or non-pregnancy. What has just been said is confirmed by the exact work of Long and Evans (41) upon the albino rat, except that in this species, as in other rodents, the regular occurrence of an ovulation about one day after parturition gives rise to a third form of the corpus luteum, the so-called corpus luteum of lactation, which again is not fundamentally different from the others, but can be distinguished by differences in size and the dimensions of the lipoidal granules in the lutein cells.

Function of the corpus luteum. We may pass over the numerous older conjectures as the function of the corpus luteum, none of which found any practical confirmation until Fraenkel (16), (17), (18) began to develop the ideas which had been given him as a death-bed legacy by his teacher, the Breslau embryologist, Gustav Born. Born had noticed that well-developed corpora lutea are found only in animals which possess a genuine placentation; in the monotremes (and as he incorrectly supposed, the marsupials) there is no corpus luteum. Furthermore, the corpus luteum of the placental mammals attains its development at the very time when the embryo is becoming attached to the uterus. Born suggested therefore that the corpus luteum is the source of an internal secretion by which the uterus is prepared for the reception and imbedding

of the ovum. At the time of Born's reflections upon this hypothesis there was only one available observation which suggested the actual existence of such a preparation of the uterus for implanting the ovum, namely, that in those animals which possess a placentation characterized by true decidua formation (man and rodents) the production of decidual cells from the uterine stroma is begun almost in the first days of pregnancy or by the beginning of the second week. It had been more or less taken for granted that the inception of the decidual changes was due to a stimulus arising from the embryo itself; but Born pointed to the occurrence of decidual changes in the uterus in tubal pregnancy and to other facts which seemed to him to indicate the dependence of the decidual preparation upon some outside influence, supposedly the corpus luteum.

Fraenkel, in planning an experimental test of Born's hypothesis, chose to use the rabbit, since at that time the early embryology of this species had been more closely followed than that of any other mammal. It was clearly known that attachment of the embryo occurs about seven days after ovulation, and that the placentation is of a decidual type. There was no further information about anatomical changes in the rabbit's uterus, serving to facilitate implantation; but assuming such a mechanism, then according to Born's hypothesis removal of the ovaries or of the corpora lutea alone during the critical six days between ovulation and implantation should prevent the attachment of the embryos. In some of Fraenkel's experiments both ovaries were ablated, with control by removal of one ovary alone; in others he cauterized the corpora lutea alone, controlling this procedure by similar trauma to other parts of the ovary without damage to the corpora lutea. The results uniformly confirmed the dependence of implantation upon integrity of the corpora lutea, for all the control animals of a large series became pregnant and all those from which the corpora lutea were totally extirpated failed to become pregnant. The outcome of this brilliant experiment has never been seriously questioned, and indeed was promptly confirmed by Marshall and Jolly (48) in a few experiments on dogs and rats, and by Niskoubina (56) in rabbits.

An equally important experimental step was taken in 1907 by Leo Loeb (36), when he conceived the idea that the maternal portion of the placenta (i.e., the decidual tissue) may be produced by the stimulus of the ova acting upon an endometrium sensitized for pregnancy by the action of the corpus luteum. Using the guinea pig for his experiments, Loeb exposed the uterus on the seventh day following an unfertilized ovulation (at which time the embryos, had they been present, would have

been about to attach themselves) and placed within the uterine cavity a small foreign body such as a piece of glass. Within four days the site of such a trauma was marked by growth of a tumor of the endometrium composed of decidual tissue. In its simplest form this beautiful experiment could be performed by simply scratching the endometrium at the proper time of the cycle. Loeb found that he could not produce deciduomata at other times of the cycle than the first week after ovulation, and that removal of the ovaries or cauterization of the young corpora lutea also prevented the development of the artificial deciduomata. Loeb's experiment has been confirmed for the albino rat by Frank (19), by Corner and Warren (12) and by Long and Evans (41). The last named writers have given a full analysis of the physiological conditions necessary for successful performance of the experiment in the rat. Gasbarrini (20), Biedl, Peters and Hofstätter (5) and Hammond (24) have also found no difficulty in repeating it in the rabbit. Kraintz (32) undertook the same experiment in dogs, in which the placenta does not contain decidual cells, and in one case succeeded to the extent that he produced by traumatizing the endometrium at the proper time of the cycle not a decidual tumor, but a simpler alteration of the endometrium closely similar to that which occurs in the placentation of this species. Passing on to mammals such as the ungulates, which have a still simpler placentation, with little or no maternal contribution to the placenta, we should expect no decidua formation nor any other reaction of the endometrium as a result of Loeb's experiment, but so far no test has been made in these animals.

The uterine cycle. Just after publication of Loeb's work a third fundamental addition to our ideas about the reproductive cycle was contributed from the Vienna gynecological clinic by Hitschmann and Adler (29) in a study of the histology of the human uterus. The Viennese workers were able to clear up a confusingly divergent mass of histological data by the simple procedure of studying specimens not chosen at random but classified by stages of the menstrual cycle. In brief, they found the endometrium in constant change throughout the cycle, and they described especially a period occupying a week or more previous to the onset of the menstrual flow, during which there is increasing hypertrophy and complexity of the glands, engorgement of the stroma, and conversion of the connective tissue into cells resembling the early decidual elements. These changes at their height resemble very closely the earliest decidual changes of pregnancy. Without reference to events in other mammalia, and lacking of course all knowledge as to the relation of ovulation to the

menstrual cycle in man, Hitschmann and Adler proposed as their hypothesis that the premenstrual changes represent a preparation for the reception of an ovum discharged and fertilized at some unspecified previous time in the cycle, and that actual implantation takes place in the late premenstrual stage. This work was done as a study in pathology rather than as a contribution to physiology; and although it has been repeatedly confirmed, it did not at once influence workers in the general physiology of reproduction. Indeed, what little information was then extant as to changes in the uteri of other mammalia in connection with the reproductive cycle and with the actually known progress of the ova in these species, was not in accord with Hitschmann and Adler's hypothesis. The evidence even seemed to indicate that menstruation precedes the time of ovulation. Marshall (43), (44), studying the sheep and ferret (*Mustelus putorius*), and Marshall and Jolly (47) studying the dog, had seen alterations in the endometrium during the prooestrous period, characterized by degeneration and even desquamation of the epithelium and by engorgement of the blood vessels, which in the dog went on to form hemorrhages in the subepithelial stroma and even to cause hemorrhagic discharge from the vagina; ovulation did not occur until these events were finished. Influenced by Heape's theory that menstruation in the human is homologous with prooestrus in other mammals, Marshall and Jolly considered that the degenerative changes described by them were similar to menstruation in the human, and therefore that ovulation in the human must follow menstruation; and assumed in this paper that the carnivore and ungulate uteri pass after ovulation into an inactive state, the "metoestrus" of Heape. Keller (30), however, studying in 1909 the dog's uterus anew, found after the prooestrous changes (in which he saw much less degeneration than indicated by Marshall and Jolly) a further series of postoestrous changes including hypertrophy of the glands, increased complexity of the surface foldings of the endometrium, engorgement of the stroma and hypertrophy of the stroma cells; he believed these changes to be in general similar to those seen in the human uterus by Hitschmann and Adler, and he felt that the parallelism between the species bore out the functional conception which they had imagined.

In 1910 Ancel and Bouin (2), apparently at the time unaware of Hitschmann and Adler's and of Keller's studies, began the work of placing a morphological foundation under Fraenkel's and Loeb's results by demonstrating the occurrence of actual changes in the rodent uterus concomitantly with the development of the corpora lutea. It will be

remembered that in the rabbit there is no spontaneous ovulation, and hence no formation of corpora lutea, except after coitus. Ancel and Bouin permitted rabbits to mate with males sterilized by vasectomy, and thus secured the development of corpora lutea without pregnancy. Under these conditions the uterus was found to become enlarged and hyperemic; its epithelium, both superficial and glandular, underwent mitotic proliferation, and the glands increased their complexity of ramification until there was produced a considerable alteration of texture which Ancel and Bouin interpreted as indicating a preparation for implantation. These changes were found to reach their maximum about the eighth day after ovulation, and were followed by retrogressive changes from about the tenth day onward, until at the twenty-fifth day the uterus had returned to the resting condition.

Hill and O'Donoghue (28) studied the reproductive cycle of a marsupial, *Dasyurus viverrinus*, in which there occurs once each year a period of oestrus followed by a spontaneous ovulation. They found a series of changes in the uterus, beginning even before oestrus and continuing after ovulation, characterized by hypertrophy and hyperemia of the endometrium, with increase of its vascularity and proliferation of the superficial and glandular epithelium. Since these changes occurred whether or not the animal became pregnant, Hill and O'Donoghue were led to consider them a mechanism for preparing the uterus for implantation, following upon an ovulation and formation of the corpora lutea; they applied the name of "pseudopregnancy" to the state brought about by the action of this mechanism in the absence of fertilization.[4]

About the same time Leo Loeb (38) described the uterine cycle of the guinea pig, which he had previously found to ovulate spontaneously every fifteen to nineteen days. He found that in this animal oestrus is accompanied by a migration of leucocytes from the uterine blood vessels through the epithelium, with the occurrence of vacuolar degeneration in the epithelial cells. Following this there is a wave of mitosis in the superficial and glandular epithelium and in the stroma, which reaches its height and ceases about the sixth day, or just before the time at which Loeb had previously found the endometrium sensitized for the production of deciduomata. These changes are identical with those of the first week of pregnancy. If the young corpora lutea are excised, the endometrial changes are inhibited until a new ovulation takes place. The cycle of the guinea pig is therefore an alternation of

[4] It seems preferable to avoid the suggestion of falsity or imitation inherent in the prefix *pseudo*, by using the terms *pregravid*, *progestational*.

uterine activity and retrogression, following the cycle of the corpora lutea.

In 1917 Marshall and Halnan (46) took up the events of the dog's cycle once more and followed it into the postoestrous stage, confirming and extending the description, given by Keller (30), of endometrial growth changes following ovulation; they showed moreover that the development and retrogression of these changes are correlated with the cycle of the corpus luteum. In this paper Marshall dropped his former theory of a homology between prooestrus and menstruation in favor of a view which assumes that in some way menstruation represents both a prooestrous and a postoestrous degeneration condensed into one.

The same year saw the publication of an important account of the guinea pig's cycle by Stockard and Papanicolaou (74), who called attention to the occurrence, at the time of ovulation, of a series of histological alterations in the epithelium of the reproductive tract, especially in the uterus and vagina. In the vagina there is first a desquamation of large quantities of epithelial cells, and then a diapedesis of leucocytes into the lumen, so that by the observation of smears made from the vaginal secretion it is readily possible to follow the cycle and to determine the day of ovulation. In the uterus there are at this time vacuolar degenerations of some of the epithelial cells, going on even to desquamation of cells or patches of epithelium; leucocytes migrate from the endometrial vessels and there may be small hemorrhages into the lumen. We are still uncertain as to the physiological significance of these changes in the vagina and endometrium. Stockard and Papanicolaou thought them to resemble human menstruation; but whether or not this interpretation is correct, the vaginal changes serve to give us for the first time a means of following the individual cycles of living rodents, and hence are already opening up in American laboratories a promising field of research. The recent studies of Evans and Bishop (15) on the relationship of diet to the ovulation cycle indicate the possibilities afforded by Stockard and Papanicolaou's discovery.

Since very little is known about the actual histology of the uterus during the first days of pregnancy in any species, the histological workers have in general merely assumed that the postoestrous changes which they have observed are actually in the nature of adaptations for implantation. The reviewer (11) undertook to test this point for one species at least, choosing the domestic pig because of its very simple placentation. The work involved following in exact detail the ovarian cycle, the progress of the ova and early embryos, and the histological changes of the uterus

during the cycle and in early pregnancy. The results were so clear-cut as to serve almost as a diagram of the mammalian reproductive cycle as we are now beginning to understand it. In this species oestrus occurs at intervals of twenty-one days. Prooestrous enlargement of the follicles is accompanied by changes in the endometrium practically similar to those described by Stockard and Papanicolaou; during the development of the follicles and then of the corpora lutea there is a wave of cell division in the endometrium; then as the early embryos are being transported and spaced within the uterus the epithelial cells assume a high columnar arrangement, as if entering a secretory phase. Next there is a stage in which the epithelium is roughened upon its surface by the extrusion of cytoplasmic processes; this stage lasts exactly as long as the period required for the actual attachment of the chorions. Up to this point (the fifteenth day) the process is the same whether or not the sow has been impregnated, but if she is not pregnant the corpus luteum suddenly retrogresses and with it the uterine epithelium returns without obvious destruction to the prooestrous stage as new follicles develop. There are parallel changes in the glands, but on the whole the alterations are mostly in the epithelium, probably because the pig has a diffuse superficial placentation; in mammals with more complex placentation the stroma and glands of the uterus are called upon for a greater share in the pronidational changes. An almost exactly similar cycle in the cow has since been described by Zietschmann (81), although without data as to the progress of the ova and embryos.

The studies of Long and Evans on the albino rat (monograph (41) in 1922 after numerous preliminary studies) are of extraordinary fulness and interest. By application of Stockard's methods they have determined the rat's cycle to be of but four to six days' duration. During this time there is a periodic alternation of histological changes in the uterus and vagina, following the maturation of follicles and the development of corpora lutea; but the frequent renewal of ovulation seems to cut short the full development of these changes. However, if a copulation occurs, the next ovulation is inhibited, and the corpora lutea persist without interruption; in this case the genital tract undergoes the alterations of pregnancy. If copulation be imitated by the insertion of a glass rod into the cervix uteri at the time of oestrus, then the same inhibition of ovulation occurs, and the genital tract undergoes alterations resembling those seen in pregnancy. This statement is based chiefly on Long and Evans' description of the vaginal cycle; they have not yet followed the uterine changes in the period following artificial prolongation of the cycle,

but they have found that it is only in such a period that artificial deciduomata can be regularly produced by Loeb's experiment. The rat's cycle as worked out by these authors represents therefore a highly specialized speeding up of the ovulation rate, with the development of a peculiar mechanism by which, in the event of mating, time is nevertheless provided for the development of the same series of post-oestrous physiological reactions which is seen in other mammalia.

Other mechanisms of the cycle. Recent investigations have begun to reveal the existence of other mechanisms at play in the complicated process of reproduction. There is a cycle of histological change in the Fallopian tube, long suspected, but not fully studied in systematically dated material. Snyder (71) has recently followed the cycle of the pig's Fallopian tube, and has described a sequence of changes in the epithelium parallel with the ovarian cycle. Observations of Moreaux (55) and others on the rabbit's tube indicate the occurrence there of still more elaborate processes of secretory nature, probably concerned with producing the mucoid coating with which the ovum is provided in this species. Another important field seems to be opening out of the work of Blair (6), Keye (31) and Seckinger (68), who have discovered cyclic variations in the spontaneous activity of the uterine and tubal musculature. As Keye and Seckinger have traced them in the pig, these variations follow with impressive regularity the cycle of the ovary. A strip of the tubal or uterine wall, suspended in oxygenated Locke's solution, continues to undergo regular contractions in vitro. Since these contractions change in rate and amplitude throughout the cycle, the resultant curve is characteristic for each stage. For instance, a particular type of contraction wave, characterized by an undulating alternation of large and small excursions, is obtained from the tube when the ova are in passage through the tubal canal and from the uterus a few days later when the degenerating ova or early embryos are in the uterine cavity. The significance of this contraction cycle can only be conjectured; Keye and Seckinger suggest the possibility that it represents a mechanism for transportation of the ova and spacing of the embryos in the uterus. The reviewer, with Seckinger, has in preparation the description of an exactly similar cycle in the tube of the rhesus monkey.

Through the studies of Ancel and Bouin (3) it is known that there are proliferative changes in the mammary gland parallel with the development of the corpus luteum. This work done on the rabbit has been confirmed by Hammond and Marshall (25). In species with spontaneous ovulation, and hence with periodic new formation of corpora lutea, it

appears that the mammary gland participates in each cycle, by vascular congestion and proliferation of its acini (39), (40). No doubt a similar process is responsible for the premenstrual mammary discomfort, common in women.

In spite of their many gaps and differences the contributions which we have reviewed have led us to a clear physiological conception of the reproductive cycle. Diagrammatically expressed, the growth of the follicle is accompanied by sexual activity and by prooestrous changes in the genital tract; after ovulation the corpus luteum brings about the progestational alterations in the tube, uterus and mammary glands. Omission of pregnancy and consequent early retrogression of the corpus luteum is followed in some animals by a long resting stage (anoestrus), in others by a brief interval (dioestrus), in still others by premature onset of a new ovarian cycle. In most animals these mechanisms occur in full at each ovulation; in others, as the rat and rabbit, there are special modifications by which the events of the progestational period are cut short. This conception, pieced together from many species by various means, is at the moment passing from the status of hypothesis to that of demonstrated fact; but much remains obscure. The post-oestrous modifications are perhaps most clearly comprehensible and subject to correlation; at least the differences between species are theoretically explainable by known differences in placentation. A greater problem is presented by the prooestrous changes of the uterus. It happens that those species about which several observers agree in the description of vacuolar degenerations, leucocytosis and mitosis in the epithelium at this stage, are all animals with rapid cycles, in which new follicles ripen at once after degeneration of the corpora lutea; thus it is not clear which of the prooestrous changes are correlated with the retrogressing corpus luteum and which with the new follicle and oestrus. The importance of this matter is obvious in connection with menstruation.

Finally, it will be seen that our information is chiefly in the nature of a chronological correlation between the outward events of the cycle, the ovarian and uterine changes, and the movement and implantation of the ova. The actual causal relationships, endocrinal or otherwise, between the follicle and corpus luteum on one hand and the uterus on the other, are as yet largely unexplored.

II. The human cycle. In the human cycle there is no sharply defined oestrual period to serve as a clue to the moment of ovulation, and moreover the occurrence of menstruation adds a complicating factor

present only in the higher primates. These differences, and the difficulties which hamper study of reproduction in our own species, have left us up to the present with a very incomplete knowledge of the menstrual cycle. Menstruation itself is variously thought (*a*) to be an independent or interpolated event with no relation to ovulation; (*b*) to represent oestrus; (*c*) to be a prooestrous event; (*d*) to be the retrogressive stage of the human cycle, after preparation of the endometrium for an ovum which does not become implanted. The heart of the difficulty obviously lies in our ignorance of the time-relation of ovulation and implantation with respect to menstruation. We do not know, by any direct observation, when the primate ovum is discharged; the unfertilized ovum has never been observed after leaving the ovary, nor has the fertilized egg been seen early enough to give us any information in regard to the earliest stages of implantation, i.e., during the first two weeks. Numerous efforts have been made to estimate the rate of ovulation with reference to menstruation from human embryos of the third week and later. The age of the embryo is estimated by one means or another, and the date of ovulation is calculated backward from this. Such conjectures have engaged some of the most eminent embryologists (His, Mall, Teacher and Bryce, Grosser, Von Spee, Triepel)[5] but the results have variously placed ovulation at any and every stage of the cycle. The method is essentially a begging of the question, and is furthermore dependent upon scanty material and takes too little account of embryonic retardation and abnormality.

It is sometimes possible to obtain in connection with a human embryo or child an exact record of the copulation date. The date of ovulation calculated from such a record would of course in the other mammalia be the same, within a day or two, as the copulation date, since these animals mate only near the time of ovulation; but in man the calculation of embryonic age from a single copulation is far from certain and indeed is often in disagreement with other data or the probabilities of the case. The great war gave to German obstetricians a singular opportunity to study the relation of conception to the menstrual cycle. Many pregnancies of soldiers' wives dated from brief furloughs of their husbands from the front. Some thousands of such cases were collected by Siegel (70) and Pryll (58) and others (see also Zangemeister (80)), and when assembled gave curves showing that conception may result from mating at any stage of the cycle, except that the last six or seven days preceding menstruation are practically sterile; fertility is highest about one week

[5] For literature of this subject see Mall (42) and Triepel (75).

after menstruation and falls at first slowly and then rapidly as the last week of the intermenstrual period approaches. To explain such a result we must needs assume that something is different from the state of affairs in other mammals; either ovulation may occur at any time of the menstrual cycle, or else the germ cells, spermatozoa or ova, or both, are able to survive for a week or two in the female reproductive tract and then take part in zygosis.

More light has been won by direct study of the ovaries. A mass of earlier work, which we shall pass by, was based on the ancient hypothesis, still widely current among the laity, that menstruation is homologous with oestrus; but among the medical profession this notion has largely given place to the view of Leopold and his colleagues (33), (34), (35), who gathered together a large number of human ovaries from operations and attempted to correlate the appearance of the corpus luteum with the menstrual history. These workers made their determinations by macroscopic observation, in accordance with a more or less preconceived idea of the corpus luteum as it was supposed to appear at each of the four weeks of the cycle; at the present time, although we are still largely ignorant as to the rate of organization of the human corpus luteum, we know enough of the facts as they exist in other animals to throw great doubt upon the value of Leopold's criteria for determination of the age of corpora lutea.

Leopold and his colleagues believed that they found corpora lutea of various ages at each stage of the menstrual cycle, and therefore they taught that there is no close correlation between ovulation and menstruation; the two events may coincide or may differ widely in point of time. This teaching, although sterile as regards physiological interpretation of the human cycle, has found numerous supporters, the most recent being Schickelé (64); but it should be noted that some of the authors have failed to distinguish between the two ideas (*a*) that there is no correlation between ovulation and menstruation, and (*b*) that the two events are merely not coincident. There is a tendency to assume the first of these statements upon proof of the latter alone.

In 1902 Fraenkel (16), (17), (18) began the exposition of his conception of the corpus luteum cycle. We have already recounted in the preceding section that part of Fraenkel's work which dealt with the theory and the proof that the corpus luteum is an organ of internal secretion by which the uterus is prepared for implantation of fertilized ova. This general idea was accompanied by a special theory of the human cycle, which Fraenkel briefly stated as follows: a follicle grows, ruptures and

is converted into a corpus luteum; the ovum is discharged and transported through the tube; meanwhile the uterus undergoes changes preparatory to nidation of the embryo, by which it attains the premenstrual type of structure. The premenstrual stage of the human is thus akin to the post-oestrous stage of other mammals. If fertilization and implantation do not occur, the endometrium breaks down and menstruation is the result. This hypothesis, foreshadowed by the older conjectures of Aveling, Sigismund and Beard, had the initial merit of proposing a definite physiological idea subject to precise investigation. It was to find almost immediate support in the histological observations of Hitschmann and Adler which we have already quoted; meanwhile Fraenkel himself undertook to test it by renewed observations of the ovary after the method used by Leopold and others. However, his experimental work on the rabbit had taught him the dangers of macroscopic examination of the corpus luteum, and he therefore made no attempt to diagnose other than "recent" corpora lutea with fresh red tissue at the points of follicular rupture. Moreover, to avoid pathological cases he made his studies during life by direct observation of the undisturbed ovaries of women subjected to laparotomy for conditions other than those of a grave gynecological nature. He found recent corpora lutea only during the second half of the interval, and was even willing to name as the average date of ovulation the nineteenth day after the onset of four-weekly menstruation, or the ninth day before the onset of the next period. The corpus luteum he believed to reach its full development about eight days later, to begin retrogression at the onset of menstruation, and to disappear at the end of this period. These observations were confirmed by Ancel and Villemin (4), who placed ovulation however twelve days before menstruation, as well as by various pupils of Fraenkel, the latest of whom is Tschirdewahn (76). Fraenkel also attempted a physiological test of his theory by cauterization of the corpus luteum in women subjected to laparotomy for various surgical reasons. In his hands this procedure resulted in postponement of the next menstruation for three to eight weeks. Halban and Kohler (23) and others have, on the contrary, found it to bring on menstruation within a few days. Such experiments and the discussion which they have aroused regarding the exact causal relation of the corpus luteum to menstruation will hardly afford exact conclusions until we are in a position to extirpate the corpus at much more precisely dated stages.

Another group of workers, realizing the uncertainties of bare macroscopic study of the ovary, have returned to the examination of excised

organs from the operating room, attempting to apply both the histological criteria of the corpus luteum recently gained from other species, and the newer knowledge of the uterine cycle as introduced by Hitschmann and Adler. The material available for such study contains almost no specimens which would be accepted by workers dealing with laboratory mammals, but after discarding the graver lesions and the more inaccurate histories there remain a few specimens comprising both ovaries, the endometrium, and a passable menstrual anamensis. Robert Meyer and Carl Ruge II (52) were able to assemble 106 specimens, of which a portion met the above requirement. Interpreting the ovaries in accordance with R. Meyer's standardization (49) of the human corpus luteum into stages of proliferation, vascularization, maturity and retrogression, they found that there is a fairly high correlation between the stages of the corpus luteum and the stage of the endometrium. The recent corpus luteum (stage of vascularization) accompanies the early premenstrual endometrium, the mature corpus luteum accompanies the later premenstrual endometrium, and retrogression of the corpus luteum generally begins about the onset of menstruation. In their first papers Meyer and Ruge placed ovulation in the first half of the intermenstrual interval, most commonly during the first postmenstrual week, but later Ruge (62), (63) modified his view by dating ovulation between the eighth and fourteenth day after onset of the menstrual flow. Meyer and Ruge's correlation between the stages of the corpus luteum and the uterus is in general convincing, based as it is upon objective comparisons between histological preparations and in no way dependent upon individual histories; and Ruge's estimate of the date of ovulation seems, like Fraenkel's, at least an approach to the truth. It must be remembered, however, that for want of actual observation of the human ovum we are still without a starting point for determination of the maturity of follicles and the age of corpora lutea. Any dating based upon a classification of stages of the corpus luteum, such as Robert Meyer's, involves an assumed comparison with other forms, and hence adds another possible source of error to those inherent in human material from the clinic, with all its variation in age and pathological condition. The effort to calculate backward to the date of ovulation from the anatomical condition of the ovaries is thus still inaccurate, though great advance has been made over Leopold's methods. No destructive criticism is intended here, however, for it is after all the especial contribution of the modern clinical workers in this field, led by Fraenkel, by Meyer and Ruge and by Schroeder, that they have utilized as far as possible the conceptions already learned from other species.

Schroeder (66),(67) has studied about 100 cases from his own clinical material, with results which appear to the present reviewer to approximate the best that can be done with such material with methods available at present to the clinical laboratory. His descriptions of the corpus luteum are in rather close accord with the experience of those who have studied other mammals under more favorable conditions, and he appears to have done much to eliminate inaccuracies due to cycles of abnormal duration and similar causes. Schroeder concludes that rupture of the follicle takes place at about the fourteenth to the sixteenth day after onset of menstrual flow, and that the corpus luteum is a solid structure within three to five days, thus reaching maturity at about the eighteenth or the twentieth day of the four-weekly cycle. The corpus luteum may persist into menstruation, but is undergoing retrogression by the end of the flow. Just as described by Meyer and Ruge, the interdependence of the corpus luteum and the endometrium is very close, the mature corpus always being found in association with a premenstrual endometrium.

The gradually increasing accuracy of such studies has suggested the possibility of more precise examination of the primate ovary along experimental lines. Reusch (60), assuming the occurrence of ovulation about the middle of the interval, took advantage of two laparotomies done upon women who were menstruating regularly, to examine the ovaries on the thirteenth and the eleventh day, respectively, preceding the calculated onset of menstruation. In each case he found and excised a corpus luteum which proved on section to be comparable with the earliest stages of the corpus luteum as found in other mammalia. Unfortunately there was no opportunity to study the endometrium.

The present reviewer has in preparation the preliminary report of an experimental attempt, now in progress, to establish the ovulation cycle of a primate, *Macacus rhesus*. This familiar monkey undergoes menstrual phenomena to all appearances quite like the human, with a cycle averaging about four weeks. During the present year it has twice been possible to discover the ovum in progress through the reproductive canal. These are the first authenticated unfertilized ova of primates yet seen after discharge from the ovary. In one case the animal was killed about the twelfth day before the onset of the next expected menstruation; the ovary contained a recently ruptured follicle, and the mature ovum was found in the corresponding Fallopian tube. The endometrium closely resembled the late interval stage of the human as classified by Hitschman and Adler. The second macaque was killed about the seventh day

before the calculated onset of menstruation; there was a large solid and mature corpus luteum, and the ovum, in a state of moderately advanced degeneration, was found in the uterine cavity. The endometrium was in an early premenstrual stage. Examination of the ovaries at other periods of the cycle showed a relation of follicles and corpora lutea to the endometrial cycle which bore out, at least in fully mature animals, the correlations of Meyer and Ruge and of Schroeder.

It will be seen that by these studies of the primate cycle, incomplete as they are, we have advanced beyond the conjectures which still occupy much of the literature, and that we cannot be far from the truth about the time of ovulation and the growth of the corpus luteum. There is less to say as yet about the other phase of the human cycle; that is, menstruation and the retrogression of the corpus luteum. The exact function of the menstrual hemorrhage is at present in doubt. The original view of Fraenkel (at least as interpreted by his earlier readers) that the corpus luteum by its activity brings on menstruation, has by almost common consent been reformulated to propose that it is the retrogression of the corpus luteum which causes breakdown and hemorrhage from the endometrium, no longer subject to anabolic stimuli from the lutein cells. If the ovum be fertilized, it is assumed that the embryo causes persistence of the corpus luteum, which in turn continues its progestational effect upon the endometrium, and thus postpones menstruation. Menstruation therefore (though by no means an abortion of the unfertilized ovum, as it is sometimes put) is on this theory merely a violent demolition of the premenstrual uterine edifice, some days after the expected tenant fails to arrive. The only normal outcome of an ovulation is pregnancy, and the hemorrhage is viewed as a pathological result of failure in this respect. I have mentioned on the other hand Schroeder's findings that the corpus luteum at least sometimes does not begin degeneration until after the onset of menstruation. One or two trustworthy human specimens in the author's small collection also show the corpus luteum still undegenerated (as far as can be revealed by the ordinary staining technique) on the first days of menstruation. It is, I believe, not beyond conjecture that the pronidational preparation of the endometrium goes actually to the verge of menstruation. We are of course ignorant as to the exact relation of human implantation to the cyclic changes of the ovary and uterus: the most valuable observation yet at hand is given by Grosser (21), in a spectacular photograph of the Kleinhans specimen, which shows a very young human embryo in the earliest stage of implantation, upon an endometrium which is typically

premenstrual. Decidual alteration of the stroma cells is just beginning, and there are a few red blood cells in the stroma, but no large extravasations. It has also been noted in connection with a number of the earlier human embryos (at stages slightly later than the Kleinhans ovum) that the endometrial glands contain blood even at a distance from the invading trophoblast (cf. Von Möllendorff (54)). In many pregnancies there is even a trace of vaginal hemorrhage at the first lapsed menstrual period. My conjecture is thus that in order to provide for the highly specialized embryonic implantation of primates, with its opening of the maternal blood vessels into the intervillous spaces, the endometrial process is carried so far as even to cause bleeding into the tissues during the last days of the interval, at the time during which the early embryo is to be implanted. The action of the embryo alters the latter part of the process so as to inhibit or limit the hemorrhage; but if no embryo be present to utilize the extravasation, then the blood escapes into the uterine lumen and externally visible bleeding occurs. Such a hypothesis seems to render the menstrual process something more than a pathological wastage, and also suggests a reason for its total absence in species which have less specialized forms of implantation: but there can be no solution of these queries until in man or some other primate we possess a sufficient series of embryos of the second week to give us facts instead of hypotheses.

Without exact knowledge we are also unable to deal fully with a doctrine which is having its latest recrudescence in the works of R. Meyer (50), (51) and others,—the theory of "primacy of the ovum,"—which assumes that it is the egg-cell itself which by its growth brings on through some hormonal effect the positive phase of the menstrual cycle and by its death when not fertilized causes destruction of the endometrium. The reviewer can add little to the objections already leveled at this hypothesis by Seitz (69) and by Evans (14), except to refer once more to his own description of a monkey in which the ovum was actually found in degeneration while the endometrium was as yet premenstrual. There is every reason to suppose that in man and the monkey as in other mammals the unfertilized ovum has entirely disintegrated long before completion of the progestational stage of the uterus. An ovum which is non-existent can surely not long affect the uterus. We have, no doubt, a long time to wait for chemical identification of the reproductive hormones and their exact sources; meanwhile the burden of proof is upon those who discount the physiological possibilities of the general ovarian apparatus, including especially the follicles and the corpus

luteum, in favor of so minute and detached an object as the mammalian egg.

In summary, it may fairly be stated that the trend of recent work is to suggest the basic similarity of the primate reproductive cycle to that of the other mammalia as outlined in the first section of this review. We have good reason to believe in the occurrence of periodic maturation and discharge of the Graafian follicles at a time near the middle of the interval (in the typical four-weekly cycle) or ten to fifteen days preceding the onset of menstruation. The corpus luteum probably reaches maturity in a few days, as in other species, and persists throughout the last week of the interval, to begin its retrogression about the time of menstruation. If the ovum is not fertilized, it degenerates in the uterus a few days after its discharge (a conjecture for which we have as evidence, as far as primate species are concerned, only the one monkey's ovum mentioned above); but if fertilized, it reaches the uterus during the stage of premenstrual alteration of the endometrium which accompanies the mature corpus luteum. At this time we may suppose the uterine stroma is sensitized for the production of decidual cells and is ready to receive the embryo. If fertilization and imbedding do not occur, the premenstrual endometrium proceeds to the stage of menstrual hemorrhage.

In adopting for the purposes of further study so satisfactory an explanation of the primate cycle, we must admit that it does not meet, without additional conjecture, a difficulty presented by the accepted belief that the human female is fertile at all times of the cycle, except possibly during the last week of the intermenstrual period as shown by Siegel and others. This difficulty can be theoretically explained in several ways. Granting that ovulation is usually regular and narrowly limited to a particular time of the cycle, there may be either extra-cyclic ovulations, possibly induced by sexual stimulation, as suggested by Triepel (75) and Evans (14), or else the germ-cells are able to survive in the female reproductive tract for as much as a fortnight. In other mammals (so far as known) the unfertilized ova begin to degenerate by the end of their three-day sojourn in the tube. As to the possible survival of spermatozoa in the human Fallopian tube, we have conflicting reports. Observations on other mammals with limited oestrous periods are obviously irrelevant, since mating occurs only at the time of ovulation, and survival of spermatozoa would be useless. The question needs serious investigation. Meanwhile it may be said that if we accept a regular ovulation occurring about twelve days before onset of menstruation, and assume the viability of spermatozoa in the oviduct for a space

of two weeks following coitus, then all the known interrelations of the human reproductive cycle can be seen to follow. Finally there is the bare possibility that if all causes of error could be ruled out of such statistics as Siegel's the human female might actually be found to be fertile only during a limited portion of each cycle near the day of ovulation.

The whole story illustrates anew the interdependence of physiological and morphological methods. Without the classical labors of von Baer and a long succession of embryologists we should not have had even a clue to this grave question of human physiology; in a later period of the investigation we have again seen the histologist point the way, but only after he had learned to classify his material by functional stages. In the end, the problem as it especially concerns our own species will be solved by taking into the clinic the great advances of method now in the making by physiological anatomists.

BIBLIOGRAPHY

Among recent reviews of this subject, the following may be especially mentioned:

Evans, H. M. The rhythm of gonadal function, with especial reference to the relations between uterus and ovary. In: Barker, Hoskins and Mosenthal: Endocrinology and metabolism, New York, 1922.

Loeb, L. The relations of the ovary to the uterus and mammary gland from the experimental aspect. Surg., Gynec. and Obstet., 1917, xxv, 300.

Marshall, F. H. A. The physiology of reproduction. 2nd ed., London, 1922.

Zietschmann, O. Ueber Funktionen der weiblichen Genitale bei Säugetier und Mensch. Arch. f. Gynäkologie, 1922, cxv, 201.

(1) Allen: Amer. Journ. Anat., 1922, xxx, 297.
(2) Ancel and Bouin: Journ. de Physiol. et Path. gen., 1910, xii, 1.
(3) Ancel and Bouin: Journ. de Physiol. et Path. gen., 1911, xiii, 31.
(4) Ancel and Villemin: C. R. Soc. Biol. Paris, 1907, lxiii, 200.
(5) Biedl, Peters and Hofstätter: Zeitschr. f. Geb. u. Gynäk., 1921, lxxxiv, 59.
(6) Blair: Anat. Rec., 1922, xxiii, 9.
(7) Cesa-Bianchi: Internat. Monatschr. f. Anat. u. Physiol., 1908, xxv, 1.
(8) Clark: Johns Hopkins Hosp. Repts., 1900, ix, 593.
(9) Corner: Pub. Carnegie Inst. Washington, 1915, no. 222, 69.
(10) Corner: Amer. Journ. Anat., 1919, xxvi, 117.
(11) Corner: Pub. Carnegie Inst. Washington, 1921, no. 276, 117.
(12) Corner and Warren: Anat. Rec., 1919, xvi, 168.
(13) Drips: Amer. Journ. Anat., 1919, xxv, 117.
(14) Evans: In Barker, Hoskins and Mosenthal, Endocrinology and metabolism, 1922, ii, 580.

(15) Evans and Bishop: Journ. Metab. Research, 1922, i, 319, 385.
(16) Fraenkel: Arch. f. Gynäk., 1903, lxviii, 438.
(17) Fraenkel: Arch. f. Gynäk., 1910, xci, 705.
(18) Fraenkel: Zeitschr. f. Geb. u. Gynäk., 1913, lxxiv, 107.
(19) Frank: Surg., Gynec. and Obstet., 1911, xiii, 36.
(20) Gasbarrini: Inter. Monatschr. f. Anat. u. Physiol., 1911, xxviii, 259.
(21) Grosser: Zeitschr. f. Anat. u. Entwick., 1922, lxvi, 179.
(22) Guttmacher and Guttmacher: Johns Hopkins Hosp. Bull., 1921, xxxii, 394.
(23) Halban and Kohler: Arch. f. Gynäk., 1914, ciii, 575.
(24) Hammond: Proc. Roy. Soc. London, 1917, lxxxix B, 534.
(25) Hammond and Marshall: Proc. Roy. Soc. London, 1914, lxxvii B, 422.
(26) Hartman: Journ. Morphol., 1919, xxxii, 1.
(27) Heape: Quart. Journ. Micr. Science, 1900, xliv, 1.
(28) Hill and O'Donoghue: Quart. Journ. Micr. Science, 1913, lix, 133.
(29) Hitschman and Adler: Monatschr. f. Geb. u. Gynäk., 1908, xxvii, 1.
(30) Keller: Anat. Hefte, 1909, xxxix, 309.
(31) Keye: Johns Hopkins Hosp. Bull., 1923, xxxiv, 60.
(32) Kraintz: Arch. f. mikr. Anat., 1914, lxxxiv, 122.
(33) Leopold: Arch. f. Gynäk., 1883, xxi, 347.
(34) Leopold and Mironoff: Arch. f. Gynäk., 1894, xlv, 506.
(35) Leopold and Ravano: Arch. f. Gynäk., 1907, lxxxiii, 566.
(36) Loeb: Centralbl. f. allg. Pathol., 1907, xviii, 563.
(37) Loeb: Journ. Morphol., 1911, xxii, 37.
(38) Loeb: Biol. Bull., 1914, xxvii, 1.
(39) Loeb and Hesselberg: Journ. Exper. Med., 1917, xxv, 285.
(40) Loeb and Hesselberg: Journ. Exper. Med., 1917, xxv, 305.
(41) Long and Evans: Mem. Univ. California, 1922, vi.
(42) Mall: Amer. Journ. Anat., 1918, xxiii, 397.
(43) Marshall: Phil. Trans. Roy. Soc. London, 1904, cxcvi B, 47.
(44) Marshall: Quart. Journ. Micros. Science, 1904, xlviii, 323.
(45) Marshall: The physiology of reproduction, 2nd ed., London, 1922.
(46) Marshall and Halnan: Proc. Roy. Soc. London, 1917, lxxxix B, 546.
(47) Marshall and Jolly: Phil. Trans. Roy. Soc. London, 1906, cxcviii B, 99.
(48) Marshall and Jolly: Phil. Trans. Roy. Soc. London, 1906, cxcviii B, 123.
(49) Meyer: Arch. f. Gynäk., 1911, xciii, 354.
(50) Meyer: Arch. f. Gynäk., 1913, c, 1.
(51) Meyer: Arch. f. Gynäk., 1920, cxiii, 259.
(52) Meyer and Ruge: Zentralbl. f. Gynäk., 1913, xxxvii, 50.
(53) Miller: Arch. f. Gynäk., 1910, xci, 263.
(54) Von Möllendorff: Zeitschr. f. Anat. u. Entwick., 1921, lxii, 353, 406.
(55) Moreaux: C. R. Soc. Anat., 1911, 3me Sess., 159.
(56) Niskoubina: Thesis, Nancy, 1909.
(57) Prenant: Rev. gen. des Sciences, 1898, ix, 646.
(58) Pryll: Münch. med. Wochenschr., 1916, lxxxiii (2), 1579.
(59) Regaud and Policard: C. R. Soc. Biol. Paris, 1901, liii, 470.
(60) Reusch: Arch. f. Gynäk., 1916, cv., 262.
(61) Robinson: Trans. Roy. Soc. Edinburgh, 1918, lii, 302.
(62) Ruge: Arch. f. Gynäk., 1913, c, 20.

(63) Ruge: Arch. f. Gynäk., 1918, cix, 302.
(64) Schickelé: Gynec. et Obstet., 1921, iii, 170.
(65) Schochet: Surg. Gynec. and Obstet., 1920, xxxi, 148.
(66) Schroeder: Arch. f. Gynäk., 1914, ci, 1.
(67) Schroeder: Arch. f. Gynäk., 1915, civ, 27.
(68) Seckinger: Johns Hopkins Hosp. Bull., 1923 (in press).
(69) Seitz: Arch. f. Gynäk., 1922, cxv, 1.
(70) Siegel: Zentralbl. f. Gynäk., 1921, xliv, 984.
(71) Snyder: Johns Hopkins Hosp. Bull., 1923, xxxiv, 121.
(72) Sobotta: Arch. f. mikr. Anat., 1896, xlvii, 261.
(73) Sobotta: Anat. Anz., 1914–15, xlvii, 448, 602.
(74) Stockard and Papanicolaou: Amer. Journ. Anat., 1917, xxii, 225.
(75) Triepel: Anat. Anz., 1919, lii, 225.
(76) Tschirdewahn: Zeitschr. f. Geb. u. Gynäk., 1921, lxxxiii, 80.
(77) Van der Stricht: Arch. de Biol., 1912, xxvii, 585.
(78) Völker: Arch. f. Anat. u. Entwick., Jahrg. 1905, 301.
(79) Winiwarter and Sainmont: Arch. de Biol., 1909, xxiv, 627.
(80) Zangemeister: Arch. f. Gynäk., 1917, cvii, 405.
(81) Zietschmann: Arch. f. Gynäk., 1922, cxv, 201.

THE FILTERABLE VIRUSES

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The term "filterable viruses" was introduced in the late nineties of the preceding century, to denote a group of disease producing agents, which seemed to differ from other forms of living matter in their ability to pass through earthenware filters having a pore diameter smaller than the smallest bacteria then known. They were discovered at a time when the inability to demonstrate or isolate the causative agents of many infectious diseases affecting man and animals had raised the question of the possible existence of ultramicroscopic organisms.

Inasmuch as none of the viruses, in the early years of their discovery, had been demonstrated with the microscope, the term ultramicroscopic organisms was used synonymously with the term filterable viruses. Following the discovery by Borrel, v. Prowazek, Lipschuetz, Volpino, Paschen and others, however, that in a number of diseases the causative filterable agent can be rendered visible by suitable methods of concentration and staining, the term ultramiscroscopic organism was abandoned, even though many of the filterable viruses still remain in the domain of the invisible. Physicists have expressed the opinion that these are not ultramicroscopic, however, in the sense that their minute size renders them invisible, but that they have escaped detection for want of a suitable method of illumination. As a matter of fact no representative of the filterable viruses has yet been demonstrated with the ultramicroscope which could not also be seen with the usual high power lenses in the dark field. For practical purposes then the term filterable viruses may be retained, even though we are conscious that the group in all probability is not a homogeneous one.

The first representative of the filterable viruses was discovered in 1892 by Iwanowski (1) in the course of his investigations into the etiology of the mosaic disease of tobacco. He found that he was able to produce the disease in healthy plants by inoculating them with the filtered juice obtained from diseased leaves. He also established the remarkable viability of the filtered virus, which remained active for ten months. This characteristic is shared by many representatives

of the group, and in itself seems to differentiate them from the common disease producing bacteria and protozoa.

In 1898 Beijerinck (2) published the results of his observations on the etiology of the same disease, evidently in ignorance of Iwanowski's earlier work, arriving at similar conclusions.

In the same year there also appeared Loeffler and Froschs' (3) epoch-making studies on the etiology of hoof and mouth disease. These investigators found that they could produce the disease in question by inoculating animals with Berkefeld filtrates of the contents of the specific vesicles, and that the disease could thus be transmitted in passage ad infinitum. They fully realized the basic importance of their results, and suggested that other infectious diseases of hitherto unknown etiology, such as variola, vaccinia, scarlatina, measles, Rinderpest, etc., might be due to organisms of this order. Their predictions have been amply fulfilled, for in his collective survey of the subject, published in 1913, Lipschuetz (4) could present a list of forty-one diseases affecting man and various animals, in which the filterable nature of the causative agent had been established with more or less certainty. Of human diseases this list includes yellow fever, rabies, molluscum contagiosum, dengue, warts, pappataci fever, variola, trachoma, epidemic parotitis, poliomyelitis, scarlatina, measles, alastrim and inclusion blenorrhea, while of animal diseases the following are represented: hoof and mouth disease, pleuropneumonia of cattle, South African horse sickness, chicken plague, epithelioma contagiosum, sheep pox, Rinderpest, hog cholera, catarrhal fever of sheep, heart water disease of sheep, agalaktia contagiosa, benign papulous stomatitis of cattle, leukemia of chickens, myxomatosis of rabbits, Nairobi disease of sheep, jaundice of silk worms, Rous' filterable sarcoma of chickens, distemper of horses, besides certain epidemic diseases which have been described as affecting black birds, rats and guinea pigs.

Since 1913 a number of additional diseases have been more or less definitely linked up with the activity of filterable viruses, such as the d'Herelle disease of bacteria, the mosaic disease of tomatoes, cucumbers, lettuce, beans, etc.; further the wilt disease of the gypsy moth, the sacbrood disease of bees, and amongst diseases affecting man, paravaccinia, influenza, the various types of herpes, and possibly also epidemic encephalitis.

In the present survey it is the writer's intention to discuss in some detail certain phases of the more modern work that has been done in connection with the filterable viruses, rather than to go over the achieve-

ments of earlier workers, excepting in so far as this may be necessary for the proper understanding of the subject.

THE NATURE OF THE FILTERABLE VIRUSES. *1. The question of their corpuscular character and size.* In discussing the nature of the filterable viruses, it is necessary to bear in mind that mere filterability can hardly be regarded as sufficient grounds for assuming that the viruses represent a homogeneous group, and it is quite possible that what may be found to hold good for one or more representatives, does not hold good for others. A priori it is thus possible that some of the viruses may not be of corpuscular nature. This possibility was discussed already by Beijerinck, who expressed the belief that the virus which causes the mosaic disease of tobacco was a *contagium vivum fluidum*. He came to the conclusion that the virus was not corpuscular, but present in solution, on the basis of the following experiment. Ground up, diseased leaves were placed on thick agar plates and left for ten days. The surface was then washed off with water and a strong solution of bichloride of mercury, and the top layer of the agar removed; the bottom layers nevertheless proved to be infective. Inasmuch as Beijerinck's studies of the disease along bacteriological lines had only led to negative results, and filtration experiments with very dense porcelain filters had shown that the virus could pass through the pores, his conclusion that the infective principle was present in solution does not seem unnatural. The puzzling point, however, was that it proved possible to infect an unlimited number of leaves by starting with the minutest quantity of the virus, so that the conclusion seemed unavoidable that the virus actually multiplied in the plant. In this manner he was led to assume the existence not only of a contagium fluidum, but of a contagium *vivum* fluidum.

Later investigations made in connection with various other viruses have brought forth no evidence to suggest that any of them are not of corpuscular nature. Following the discovery on the part of Giemsa and v. Prowazek (5) that the virus of chicken plague can be held back by agar ultra filters, the application of the same or similar methods to the study of other viruses showed that all that were tested were held back. By making use of graded ultra filters, i.e., of ultra filters of varying density, it further proved possible, not only to demonstrate the corpuscular character of various viruses, but also to determine their size. Andriewsky (6) thus came to the conclusion that the virus of chicken plague must consist of aggregates which are smaller than those of hemoglobin, viz., smaller than 23 to 25$\mu\mu$. Duggar and Karrer (7)

have similarly shown that the infective particles which cause the mosaic disease of tobacco have approximately the same diameter as the hemoglobin molecule which Bechold calculated to be from 33 to 36$\mu\mu$.

In favor of the corpuscular nature of a number of viruses are also the results which have been obtained by sedimentation and centrifugation experiments. v. Prowazek (8) could thus show, in the case of the vaccine virus, that on prolonged standing the upper layers of vaccinia filtrates became non-infective or at least less infective. This experiment was thus confirmatory of an older diffusion experiment of Chauveau's. This investigator layered vaccine lymph under water, and then found after some time that the proteins and salts had diffused into the upper layers, while none of the virus could be demonstrated here.

By centrifugalizing emulsions of rabies and chicken plague containing material, for a long time and at high speed, Remlinger and Landsteiner (9), Barrett and Russ similarly found that the virus was finally present in greater concentration in the lower than in the upper part of the tube.

Quite recently MacCallum and Oppenheimer (10) further showed that it is possible to shift the position of the vaccine virus in a mixture of glycerin and Locke's solution, by changing the specific gravity of the mixture. On examining the surface layer in one instance in which the virus had been brought to the top, these investigators were able to demonstrate the presence of innumerable tiny granules, which could be stained by Giemsa's method.

2. Are the filterable viruses animate or inanimate? In discussing this question it should again be emphasized that any conclusions which may be reached in reference to the animate or inanimate nature of a given virus should not be generalized, that they hold good for the special virus under consideration, and for this only.

As has been pointed out above, Beijerinck came to the conclusion that the virus causing the mosaic disease of tobacco was of non-corpuscular nature, but he nevertheless endowed it with one of the most striking properties of living matter, viz., the power of reproduction. In spite of the fact that no one since has suggested the possible existence of a contagium vivum fluidum, in the sense of Beijerinck, the idea, nevertheless, deserves more than passing notice. Because it is possible, by starting with an infinitesimally small quantity of the virus, to serially infect an endless number of plants, Beijerinck naturally concluded that the virus must reproduce itself. But, inasmuch as it

was impossible to produce the disease in a leaf which had already unfolded, while it readily developed in leaves which were in the stage of the Anlage at the time of the inoculation, Beijerinck inferred that the virus can reproduce itself only when it is bound to the protoplasm of the cells of the Anlage. Regarding the manner in which this occurs, he offers no suggestion, however.

The question now arises, even though the concept of a contagium *vivum* fluidum cannot be upheld, whether a contagium *inanimatum* may not exist, irrespective of its physical state. In this connection I would refer in some detail to certain observations which Bauer (11) published in 1904, and which are scarcely known to animal pathologists. notwithstanding their evident basic importance. In discussing the etiology of a form of variegation affecting certain Malvaceae, which is known to plant pathologists as infectious chlorosis, Bauer pointed out that the disease in question cannot be transmitted to healthy plants by inoculation of the expressed juice from diseased leaves, but only by grafting. After a plant has once become infected, an endless number of diseased descendants may be obtained by the simple process of planting infected twigs. As in the mosaic disease of tobacco, here also only those leaves develop the malady, which were in the stage of the Anlage at the time of grafting, or shortly thereafter. Bauer now argues as follows: The disease in question is a typical infectious disease, inasmuch as it is possible to infect an unlimited number of healthy plants with grafts of even single diseased leaves. The virus must hence multiply, but it cannot be living matter itself for the reason that the disease can be transmitted by grafting only. He suggests that the virus need not multiply in the ordinarily accepted sense of the term, however, but that it may be possible that some abnormal metabolic product of the diseased leaf may function as the virus and stimulate the formation of the same product on the part of the cells in the Anlage. As a consequence leaves would develop in which the identical defect would exist, as in those with which we started, and the continued production of the same substance would affect subsequently developing Anlagen in the same way. In other words, Bauer postulates the existence of a virus of the very type that we suggested above, viz., a contagium inanimatum.

Of great interest in connection with this question, further, are the developments which have occurred in the course of the study of the so-called d'Herelle phenomenon. It will be recalled that d'Herelle (12) discovered the presence in the stools of convalescent dysentery patients

of a substance which was capable of passing through the pores of porcelain filters and, when added to an emulsion of dysentery bacilli, brought about their dissolution. On transferring a loopful of the contents of this mixture to a second tube containing an emulsion of dysentery bacilli, these also dissolved. This process could be continued indefinitely, and it appeared, moreover, that during passage the activity of the lytic principle underwent an actual increase. On the basis of these observations and further studies, d'Herelle concluded that the lytic agent must be endowed with life, as it evidently multiplied in the course of its transfers. All investigators who have studied this phenomenon were able to confirm d'Herelle's basic observations, and it was soon found that lytic bodies, corresponding to other bacteria also, existed in nature. To the entire group d'Herelle applied the generic term Bacteriophagum, and he looked upon its representatives as belonging to the class of the filterable viruses and as living organisms. To this opinion he has adhered ever since. While some investigators share his view, others are inclined to look upon the lytic principle as an inanimate agent, and as a product of the bacteria themselves. Doerr (13), Twort, Bordet and Ciuca, Gratia, (14) and others have thus drawn attention to the fact that the lytic agent increases in quantity only during bacterial reproduction, and that when this is prevented, the bacteriophageal titre of the emulsion does not rise. They have shown, moreover, that by starting with a culture of bacteria, devoid of any bacteriophageal properties, these can be made to appear by various artificial means, such as an unfavorable temperature, treatments with immune sera, the mere process of filtration through porcelain filters, etc. These observers have accordingly come to the conclusion that the d'Herelle phenomenon represents an acute infectious disease of the corresponding bacteria, which is due not to a living organism, but probably to an enzyme, furnished by the bacteria themselves, which stimulates the bacteria to renewed formation of this very enzyme, which in turn leads to the destruction of the microörganism.

Observations such as these, if confirmed by further investigations, would be of fundamental importance inasmuch as they suggest new lines of investigation of various diseases, the etiology of which is as yet unknown. We would certainly have to revise our definition of the term infectious disease, which at present implies the activity of an animate agent and the possibility of its transmission from animal to animal, either directly or indirectly. If similar conditions were operative in animals, as apparently are at work in the infectious chlorosis

of plants, various maladies, which now are vaguely classed as metabolic diseases, could be viewed with better reason as infectious processes, and the same would be true for malignant growths. The urgent need of some intensive work along these lines is thus manifest.

3. The taxonomic position of the animate filterable viruses. While the possibility cannot be ignored that some of the filterable viruses may be of inanimate nature, there is evidence to suggest that the majority of those, with which we are already more or less familiar, are living organisms. Several of these have been actually cultivated, even though with considerable difficulty, and not without serial breaks, such as the virus of the pleuropneumonia of cattle (Nocard and Roux), that of poultry diphtheria, which is now generally recognized as identical with epithelioma contagiosum (Bordet), the chicken plague virus (Marchoux, Landsteiner and Berliner), the virus of poliomyelitis (Flexner and Noguchi), Olitsky and Gates' supposed causative organism of influenza, and if recent reports are correct, the vaccine virus (Plotz). In the case of the other representatives of the filterable virus group, cultivation has not yet been achieved, but evidences of division have been obtained in corresponding granule formations, which are viewed as the virus proper. Lipschuetz, it will be recalled, suggested the term strongyloplasms for these visible viruses, in contradistinction to those which have not yet been seen with the microscope.

A question that has been asked again and again, is whether the filterable viruses are bacteria or protozoa. To this a definite answer cannot be given. From the fact that an organism can be cultivated in the test tube it does not follow that it must be a bacterium, for many protozoa can be cultivated with equal readiness. To argue for the bacterial nature of an organism on the basis of its form is likewise scarcely warrantable. The fact, therefore, that the virus of smallpox or of poultry pox appears under the microscope as a round granule, is no more evidence that the organism is a bacterium than the capability of locomotion would stamp the typhoid bacillus as a flagellate protozoon. In many viruses belonging to the group of strongyloplasms division has been noted, but a type of division which differs from that of the bacterial cocci. For whereas bacterial division proceeds by direct cleavage, strongyloplasmatic division seems always to be preceded by a gradual process of median constriction, with the consequent formation of dumbbell forms and their gradual separation.

That some of the filterable viruses are more closely related to the protozoa than to the bacteria, is suggested by their peculiar cell tropism and their evident reproduction within the cells and there only. As examples I would recall the remarkable affinity of the variola-vaccinia virus, of the virus of sheep pox, of epithelioma contagiosum, and of hoof and mouth disease for the skin; that of rabies, of poliomyelitis and of chicken plague for the central nervous system; that of pleuropneumonia, of agalaktia contagiosa, of parotitis epidemica for special organs, viz., the lung, the udder and the parotid gland. It is well known that following the intravenous injection of vaccine virus, this rapidly disappears from the circulation and becomes deposited in the skin and there only; the injection of chicken plague virus is similarly followed by its disappearance from the circulation and its deposition in the brain.

Recent investigations have confirmed earlier observations by Novy and MacNeal that certain protozoan parasites (Trypanosoma lewisi) may assume a state in which they can pass the pores of antibacterial filters. Reich and Beckwith (15) thus report that they succeeded in infecting guinea pigs with filtrates from the organs and blood of animals infected with Trypanosoma brucei.

I would further recall that yellow fever has been produced repeatedly by injecting filtrates of the diluted serum of patients. Reed, Carroll, Agramonte and Lazear (16) first demonstrated this in 1900, and on the basis of these observations came to the conclusion that the causative organism must be of such minute size as to be classed among the "ultramicroscopic" organisms. When Noguchi then suggested that his Leptospira icteroides might be the cause of yellow fever, the question arose how this could be reconciled with the demonstrated filterable nature of the virus. It was then suggested that even though the leptospira measured 4 to 9μ in length, that its breadth only amounted to 0.2μ, and that it is the breadth of an organism which determines its filterability. It was further argued that the organism could pass the filter not only because of its small, transverse diameter, but also owing to its plastic (non-rigid) structure. This may or may not be so, and until leptospiras presenting their usual size and form will have been demonstrated microscopically in the filtrates, the possibility cannot be denied that the organism may be able to assume yet another form and pass in that state.

However this may be, the writer would not suggest for a moment that because an organism, be it a protozoon or a bacterium of the

usually recognized types, can assume a filter passing form, differing from the usual, it should therefore be removed from its proper group and placed in that of the filter passers. More work, however, will have to be done, before it can be definitely accepted that organisms whose normal morphology would warrant their classification either amongst the bacteria or protozoa may under special conditions assume a form which in the past was unknown in connection with the life history of bacteria and protozoa. If further investigations should actually prove the possibility of such an occurrence, a biological explanation would have to be sought. In that event the hypothesis might be advanced that various low forms of animal and vegetable life, while becoming differentiated to higher forms, may yet retain the power to return, temporarily at least, to a more primitive state, with a reduction in size to a point where they would be able to pass the pores of the average antibacterial filter. While such a possibility has already been demonstrated for certain protozoa as shown above, there is evidence to suggest that bacteria endowed with this property also exist. Olitsky and Gates (17) have thus shown that the organism which they have described under the name Bacterium pneumosintes may assume a filter passing size, while at other times it may attain a length of one micron. The same writers have also discovered the occurrence in the nasopharynx of four other organisms of the same order which, in contradistinction to Bacterium pneumosintes, are non-pathogenic.

While Olitsky and Gates have termed the organism which they regard as the probable cause of influenza, *Bacterium pneumosintes*, they have done so on purely morphological grounds, and have left the question whether or not it really is a bacterium an open one.

To assume, on the basis of our present knowledge, that the filter passers are either bacteria or protozoa, is unwarrantable. We may say that certain observations suggest that a few protozoa, and possibly bacteria may assume a filter passing size, but so far as the great majority of the filterable viruses goes, there is no evidence whatsoever to warrant the claim that their taxonomic position has been established. It might possibly be best to classify them as protista, were it not for the fact that there is evidence to suggest that some of them may be inanimate, even though corpuscular. Theoretically there can certainly be no objection to the formulation of the hypothesis that disease producing agents of corpuscular nature may exist, which in part belong to the lowest forms of life, which are neither bacteria nor protozoa,

and in part to the higher forms of ferments, and that the main group of filterable viruses may properly find its position here rather than either amongst the bacteria or protozoa.

4. The relation between filterable viruses and specific cell inclusions. The occurrence of certain cell inclusions which had been noted in several infectious diseases, such as molluscum contagiosum, epithelioma contagiosum and variola, long before the existence of filterable viruses was even suspected, assumed a new significance when it was shown that their appearance was intimately connected with the activity of the infective agent, or what has come to be regarded as the infective agent. Borrell (18) in 1903 was the first to draw attention to the apparent connection between the presence of the infective agent of sheep pox and the presence in the lesions of cell inclusions, resembling those seen in variola, on the one hand, and exceedingly minute granules, which could be stained by the aid of Loeffler's flagellar stain, on the other. In 1907 appeared v. Prowazek's classical study (19) of the various cell inclusions which had been noted up to that time, viz., the three mentioned above, Borrel's sheep pox bodies, the Negri bodies of rabies and the trachoma bodies which v. Prowazek had discovered himself in association with Halberstaedter. On the basis of his studies of these various structures, and especially of the trachoma bodies, which at a certain stage of their development could be shown to consist of innumerable tiny little granules, v. Prowazek suggested that all these inclusions are composed of virus granules proper, surrounded by a mantle of reaction products furnished by the cell which has been invaded by the virus. To express this concept of their structure, he suggested the term *chlamydozoa* for this group of the filterable viruses. The suffix, zoa, was chosen to express his belief that the viruses in question belonged to the group of protozoa or were more closely related to these than to the bacteria. Lipschuetz (20), it will be recalled, proposed the term *strongyloplasms* for all viruses of the filterable type, which can be seen through the microscope, irrespective of their association with reaction products. That v. Prowazek's interpretation of the meaning of these cell inclusions has in the main been correct, has now been abundantly demonstrated. This discovery of course, has shown a new direction in which research might profitably be carried on in the search for as yet unknown causative agents of diseases of apparently infectious nature, for the presence of cell inclusions now suggests the activity of a filterable virus. It will be well to bear in mind, of course, that the absence of cell inclusions in a given

disease does not imply the absence of a filterable virus as the etiological agent. But it also goes without saying that even though inclusions have in the past not been noted in the specific lesions produced in the course of a disease, it does not follow that they may not be present after all, that they merely have been overlooked in the past. A great deal of reinvestigative work will have to be done in the various infectious diseases in which the cause is as yet unknown, before we can definitely exclude the activity of a filterable virus. That such a reinvestigation may be crowned by success, has already been shown. Lipschuetz (21) thus was able to demonstrate the presence of cell inclusions in the lesions of all three types of herpes, viz., herpes simplex (febrilis), herpes genitalis and herpes zoster, which prominent investigators in the past had evidently either overlooked or failed to recognize. That these inclusions are in reality specific, and that Lipschuetz's interpretation of their nature is correct, is shown by the fact that the identical structures appear in the corneal epithelial cells of the rabbit, following inoculation with the contents of the herpetic vesicles. Their presence, moreover, is confined to the actual seat of the lesions which develop as a consequence of the inoculation; in herpes simplex and herpes pro-genitalis they have been shown to appear in serial inoculations, and in the case of herpes simplex the virus has been shown to be filterable. In this connection it may be interesting to note that whereas the majority of cell inclusions appear in the cytoplasm of the affected cells, the herpetic inclusions occur practically exclusively within the nucleus. Similar inclusions, occurring both in the cytoplasm and nucleus have been found by the same observer in paravaccinia (22). The latter term has been applied to an anomalous response to vaccination with calf lymph, which so far as known has only been observed in Austria, or more specifically in Vienna. The opinion has been advanced that in some manner some of the calf lymph produced at one of the vaccine institutes of that country has become contaminated by an aberrant form of vaccine virus, and that this has been propagated together with the latter. If this be so, it is remarkable that the resultant cell inclusions should be so different from the Guarnieri bodies of vaccinia. It is true that the latter may also appear within the nucleus, but that is after all unusual.

In yet another disease of doubtful etiology cell inclusions have been described within recent years which, if confirmed, would suggest the possibility that this disease also may be due to a filterable virus. The malady in question is known as Verruga peruviana or Peruvian warts,

and apparently represents a later phase of so-called Oroya fever. The inclusions in question were discovered by da Rocha Lima (23) in endothelial cells of the corresponding lesions and are composed of minute granules, which like the majority of similar structures, can only be satisfactorily demonstrated with Giemsa's or Levaditi's method of staining.

Still more recently Gins (24) has drawn attention to the occurrence of intranuclear inclusions in the epithelial cells and cells of the underlying corium in the specific lesions which develop in guinea pigs after inoculation with the virus of hoof and mouth disease. It will be recalled that the disease in question was the first infectious disease affecting animals in which a filterable virus could be shown to be the causative agent (Loeffler and Frosch (3) 1898). Cell inclusions have in the past been reported in the skin and the corresponding lesions affecting the mucous membranes by several observers, but none of these have proven to be specific. Whether the inclusions described by Gins are specific will have to be determined by future investigations. Inasmuch as the recognition of the disease must at present be based upon the progress of the malady or on the result of animal inoculations, which means a considerable loss of time, the possibility of reaching a prompt diagnosis by microscopic examination would prove of great advantage.

RECENT INVESTIGATIONS ON THE RÔLE OF FILTERABLE VIRUSES AS DISEASE-PRODUCING AGENTS. *a. The supposed relationship of the herpetic virus to the causative agent of epidemic encephalitis.* In the foregoing section we have already pointed out that specific cell inclusions are demonstrable in herpetic lesions (21) and that this fact in itself may be regarded as implying the activity of a filterable virus as the causative agent. The proof of the correctness of this conclusion is complete so far as herpes simplex goes, while in the case of herpes zoster and herpes progenitalis the evidence, though strong, is as yet circumstantial.

Renewed interest in the etiology of this group of dermatoses was aroused by Grueter's discovery (25) that the inoculation of the rabbit cornea with scrapings from lesions of dendritic keratitis produces a violent inflammation which can be transmitted from animal to animal. Loewenstein (26) and later Luger and Lauda (27), confirmed these observations and further showed that the inoculation of the rabbit cornea with the contents of herpes febrilis vesicles, from whatever source, led to the same result. While Loewenstein did not succeed in infecting animals with material that had been passed through a

Berkefeld filter, he concluded nevertheless from certain microscopic findings that the pathogenic agent probably belonged to the chlamydozoa. Subsequent positive filtration experiments, on the part of Luger and Lauda (27), Blanc and Caminopetros (28), Levaditi, Harvier and Nicolau (29) have confirmed the correctness of this conclusion.

Aside from the occurrence of the virus in the herpetic vesicles it would seem that the same or a closely related virus may occasionally also be encountered in the saliva of normal individuals.

The discovery of the virus of herpes febrilis or, as it would no doubt be better to say, of herpes simplex, while of great interest in itself, has attracted special attention for the reason that several investigators have reported the finding of a virus in material obtained from cases of epidemic encephalitis which seems to be identical with it, or at least very closely related to it. Doerr and Schnabel (30) thus report that rabbits which had recovered from keratitis produced by the common herpes simplex virus and which had developed an immunity to reinoculation with it, were also resistant to their encephalitic strain, even when introduced subdurally. Levaditi, Harvier and Nicolau (29), as well as Blanc and Caminopetros (28) did not obtain the same, but very similar results. They found that their supposed encephalitic virus was more virulent than their herpetic virus, and that while animals which had become immune to the former were also immune to the latter, the converse did not hold good. As a matter of fact a certain proportion of rabbits which have been inoculated with the common herpes virus, after a variable length of time develop symptoms of encephalitis and usually succumb, and post-mortem lesions are demonstrable in the brains of such animals which are very similar to those observed in the human being. The idea that a filterable virus may be the cause of epidemic encephalitis was originated by Strauss, Hirschfeld and Loewe (31) in 1919. These observers reported that the virus of epidemic encephalitis will pass Berkefeld N filters, that it may be preserved in glycerin, that it can be cultivated in Noguchi's spirochetal medium; that it can be stained with Loeffler's flagellar stain and Giemsa's stain; that rabbits and monkeys can be successfully infected with cultures by the subdural route, and that the same symptoms and pathological changes can be produced with these cultures, as by direct inoculation of brain substance, naso-pharyngeal mucous membrane, spinal fluid and naso-pharyngeal secretion from human cases of the disease. They further reported that animal passage could be effected without much difficulty. Their results with naso-pharyngeal

washings were indeed so uniform that they suggested the animal experiment as a means of diagnosis.

It is noteworthy that other investigators have been far less successful than Strauss, Hirschfeld and Loewe in transmitting the supposed virus of epidemic encephalitis to experimental animals and, as matters now stand, it seems rather doubtful whether the virus of any one of the experimenters referred to above actually represents the causative agent of epidemic encephalitis.

Kling (32) in a series of recent communications, has reported that on inoculating rabbits by the subdural route with brain material from cases of epidemic encephalitis, an infection does develop which pursues a latent course, however. A few animals died after from one to three months. The remainder were killed after four to six months. In contradistinction to the findings of Doerr, Levaditi and others, Kling found changes of a more chronic character, in as much as in the meninges and in the perivascular and focal infiltrations of the brain itself, mononuclear elements predominated. The lesions, moreover, were found to affect the midbrain rather than the cortex. Kling emphasizes the identical character of the lesions in his animals with those occurring in the human being. He further points out that his virus produces no lesions in the rabbit cornea that are at all comparable with those which follow inoculation with the herpetic virus, and that no crossed immunity was demonstrable between the two types. He concludes that his virus is the true cause of epidemic encephalitis, while that of Levaditi, Doerr and their collaborators is merely the herpetic virus which has accidentally found its way to those regions where it has been found.

b. Bacterium pneumosintes and its relation to epidemic influenza. Up to the beginning of the great pandemic of influenza of 1918–1919, Pfeiffer's bacillus had been fairly generally regarded as the probable causative agent of that disease. The inconstancy of its occurrence during this epidemic, however, made the correctness of this view appear rather doubtful. The inconstant presence of various other bacteria also, which were encountered both intra vitam and post mortem, eliminated these as specific factors. The thought hence suggested itself to many observers that a hitherto undiscovered organism might be the cause of the epidemic disease, and that Pfeiffer's bacillus, like the various streptococci and staphylococci that are so frequently present, merely represent secondary invaders or symbionts. With this idea in mind Olitsky and Gates (33) undertook the study of the

naso-pharyngeal washings of cases of uncomplicated influenza with reference to the presence of such as yet unknown factors. The initial studies were begun while the epidemic wave of 1918–1919 was in progress, and have been continued up to the present time. By inoculating rabbits through the trachea with the washings from the nasopharynx of uncomplicated cases of influenza, during the first thirty-six hours of the disease, Olitsky and Gates obtained clinical reactions (fever and leucopenia, with mononuclear depression) and pathological changes in the lungs of their animals, which resembled the findings in human influenza. By injecting the lung juice from such animals into others the same results were obtained. Further investigations then revealed that the virus in question readily passed through Berkefeld V and N candles, and that when contained in bits of lung tissue, it withstood the action of a 50 per cent solution of glycerin for a period of nine months. Especially significant also was the observation that it produced an effect upon the lung tissue of rabbits which favored the invasion and infection of the lung with various bacteria, such as pneumococcus, type IV, and atypical type II, streptococci and Pfeiffer's bacillus. Attempts at cultivation showed that by starting with filtered naso-pharyngeal secretions of patients, or unfiltered or filtered lung tissue suspensions from infected rabbits growth could be obtained under strictly anaerobic conditions in a medium composed of sterile human ascitic fluid, and a fragment of fresh rabbit kidney. The organism in question, as obtained in primary culture, is a minute bacilloid body, measuring only 0.1 to 0.3μ in length, the breadth being approximately one-third of the length. In subcultures on media consisting of meat infusion—peptone broth or agar as a base, and enriched with fresh animal or vegetable tissue and especially in the presence of dextrose, larger forms up to 1μ in length may be seen.

Identical findings were obtained during the outbreak of 1920 and again during that of 1922. The clinical and pathological effect in the inoculated animals was the same whether filtered or unfiltered naso-pharyngeal washings, or filtered or unfiltered lung juice, or filtered or unfiltered cultures were used. Negative results only were gotten in the case of healthy individuals and persons not suffering with influenza, as well as with animal control material. The conclusion thus seems justifiable that the organism described by Olitsky and Gates probably represents the actual cause of epidemic influenza.

Regarding the nature of the virus in question, the writers are somewhat noncommittal. They recognize that their organism is "not of

the nature of ordinary bacteria" (IV, 728), and express the belief that it is a representative of a "group or class of minute microörganisms, which the anaerobic Smith-Noguchi technique has thrown open to exploitation." If nevertheless they termed the organism Bacterium pneumosintes they did so merely on the basis of its bacilloid form, the species name having reference to its injurious action upon the lung tissue.

In this connection it may be of interest to note that in the course of their studies of naso-pharyngeal washings, and using the same anaerobic technique, Olitsky and Gates met with a number of other filter passers which in contradistinction to Bacterium pneumosintes were non-pathogenic for rabbits.

It is clear that a very interesting field has here been opened, irrespective of the question what the taxonomic position of the organism in question may be. From the bacteria that we have known in the past, the organisms differ in at least two important particulars, viz., their minute size and the remarkable resistance which they offer to the action of glycerin. In both respects, as well as in its ease of dissemination, Bacterium pneumosintes strongly resembles the causative agent of smallpox.

c. Recent investigations into the etiology of measles. Although many attempts have been made in the past to isolate the causative agent of measles by the usual bacteriological methods, it cannot be said that any one of the various organisms which have been found, has yet been proven to be the specific cause of the disease in question. Elevation of temperature, various exanthems and enanthems and leucocytic drops have been noted following the injection of various organisms obtained from the blood and the naso-pharyngeal secretion of measles cases, but the very fact that such reactions occur inconstantly and have been obtained with different organisms, suggests that none of them can be regarded as specific. If it be remembered how readily the measles patient falls a prey to secondary invaders, notably representatives of the streptococcus family, it cannot be surprising that organisms of this order may be found, nor that corresponding antibodies should appear in the blood.

One great difficulty in the study of the etiology of measles has been that whereas blood, taken during the immediate pre-eruptive and early eruptive stage, when injected into children even in so minute an amount as 0.002 cc., produces the typical disease (34), we have not yet found an animal in which the identical clinical picture can be produced.

Significant, however, is the fact that, as Goldberger and Anderson (35) first showed in 1911, the blood and naso-pharyngeal secretion of measles patients, even after filtration through Berkefeld candles, will produce symptoms in monkeys, which at least are suggestive of the human disease. Goldberger and Anderson found that after a period of incubation, varying from three to twenty-one days, there followed a febrile period of from four to five days, and frequently an exanthem appeared three days after the onset of the fever. This varied in color from a rose red to a coppery hue and was maculo-papular in character. Scaly desquamation then followed, and after recovery the animals were immune to reinoculations. This clinical picture was transmitted through the blood through six successive groups of animals, and healthy animals contracted the disease by living in contact with the sick ones.

These results were confirmed by Nicolle and Conseil (36), Hektoen and Eggers (37) and still other investigators.

Sellard and Wentworth (38) objected to the interpretation of these findings, however, claiming, that the symptoms produced were merely symptoms of serum sickness, and that they obtained similar results by injecting normal human blood serum.

The validity of this objection falls to the ground, however, if it be remembered that Goldberger and Anderson obtained the same results in serial inoculations. Nicolle and Conseil, moreover, furnished unquestionable evidence that the clinical picture in injected monkeys is directly connected with the transfer of the virus. They injected a monkey with measles blood. After nine days the animal developed fever; two other monkeys were then inoculated with the blood of the first animal, and reacted in the same manner. At the same time a child was inoculated with the blood from the first monkey and developed typical measles. The blood of this child was injected into three monkeys, and of these two reacted with fever, as did the first.[1]

Blake and Trask (39) disposed of the validity of Sellard and Wentworth's objections in another manner. They used no blood at all, but injected naso-pharyngeal washings intratracheally. Of ten monkeys which were tested in this manner, eight developed symptoms suggestive of measles, one gave a doubtful result, and one died before the expiration of the period of incubation. The same results were

[1] It should be stated that Nicolle and Conseil's animals developed no eruption, which is contrary to the findings of Goldberger and Anderson, who noted this often, though not in all cases. Possibly this may be due to the fact that different species of monkeys were used.

obtained with the naso-pharyngeal washings, after filtration through Berkefeld N candles, and followed spraying of the nose and throat, as well as after intratracheal inoculation. The animals which recovered had become immune to a repetition of the treatment. Animal passage was also secured, so that the argument that a soluble toxin may have been responsible for the clinical picture can be ruled out.

It is to be noted that in Blake and Trask's series the clinical picture seemed to be quite constant, and it is noteworthy that both exanthems and enanthems developed, and that the histological examination of both showed the same lesions as are found in the human disease.

Inasmuch as monkey material is expensive, and the possibility of investigating problems connected with the subject of measles would thus be limited, renewed attempts have been made to infect the ordinary laboratory animals, notably guinea pigs and rabbits, with the measles virus. It was to be expected that any clinical symptoms that would be produced, would probably or at least possibly be even less characteristic and less constant in these animals than in monkeys, and for this reason some of the investigators sought to ascertain whether other effects might not be noted, which could serve as criterion of successful infection. Results of definite value have, as a matter of fact, been achieved along these lines. It has thus been shown that rabbits are quite susceptible to the action of the measles virus, both when introduced in the form of blood or of filtered naso-pharyngeal secretion, if obtained early in the course of the eruptive period.

Nevin and Bittman (40) found that in nearly all of their animals, which had been inoculated with blood, definite clinical symptoms developed between the thirteenth and seventeenth day following the inoculation, such as conjunctivitis with moderate edema of the lids, a little diarrhea, a slight exanthem, followed by desquamation in the shaved axillary and thoracic regions, and sometimes hyperemic spots on the labial mucosa. Nevin and Bittman report that in their series fever was inconstant. Especially interesting was the fact that the same results were obtained in a second series of animals, which had been inoculated with blood from the first series.

Duval and d'Aunoy (41) obtained similar results. They report, however, that the period of incubation was only of two to five days' duration; pyrexial and cutaneous as well as leucocytic reactions appeared. The rise in temperature took place on the fourth day and concomitantly with it there occurred a diminution of the total number of the leucocytes. While 90 per cent of their animals gave a pyrexial

and leucocytic response to the inoculation, only about 40 per cent developed a rash. Coryza, conjunctival injection and enanthems on the buccal mucosa of the oral cavity were common symptoms. Repeated passage through rabbits seemed to increase the virulence of the virus. A number of animals thus succumbed in the fourth and subsequent generations, and in these grave nephritic changes could be demonstrated at autopsy. A similar pyrexial and leucocytic response was obtained in guinea pigs, but in these animals the incubation period was longer, viz., nine to twelve days. Nephritis was a constant symptom. Cross passages from rabbits to guinea pigs and vice versa, were successful, and the guinea pigs which had reacted and recovered were not susceptible to reinoculation with measles blood when tested over periods of two weeks to three months after recovery.

Corresponding results were obtained by Grund (42) and Duval and d'Aunoy (41) with the filtered naso-pharyngeal secretion of measles patients. Curiously Grund, in rabbits, was unable to effect animal passage by starting with material taken from the living animals, but did succeed if the inoculations were made with the blood and lung juice of animals that had succumbed to experimental measles.

On the basis of the experimental work that has thus far been done the conclusion would seem to be justifiable that the measles virus is a filter passer, that it is present in the blood and naso-pharyngeal secretion of patients during the early eruptive stage of the disease, and that rabbits and guinea pigs may properly be used for the propagation of the virus in the laboratory and the study of its properties. Such investigations are now urgently needed, and should determine the rôle which Tunnicliff's green producing diplococcus (43) plays in the etiology of the disease.

d. Trachoma and inclusion blenorrhea. The search for the etiological agent of trachoma has been attended by great difficulties, owing in large part to the remarkable tendency of the disease to become complicated by various bacterial infections. These infections have misled various investigators into the belief that the particular organisms which they happened to encounter with some degree of regularity represented the cause of the malady. In every instance further investigations have demonstrated that the suspected bacillus or coccus was merely a concomitant organism or a secondary invader.

In 1907 v. Prowazek and Halberstaedter (44) made the discovery that peculiar inclusions may be found in the epithelial cells of the diseased conjunctiva of both human patients and orang-utangs that

had been inoculated with trachomatous material. The study of the structure of these inclusions led v. Prowazek to the development of his chlamydozoal doctrine and the conclusion that in this particular instance the constituent granules of the inclusions represented the etiological agent. Cell inclusions, in the sense of v. Prowazek, it will be recalled, signify the presence of a filterable virus which, after invading a cell, calls forth the deposition of a reaction product about the individual granules.

That the virus of trachoma is in reality filterable has been shown by Bertarelli and Cechetto (45) as well as by Nicolle, Cuénod and Blaizot (46).

Noguchi and Cohen (47) claimed to have cultivated the virus in question, but it is to be noted that they were unable to produce typical lesions with the cultures. In the same year (1913) Halberstaedter and v. Prowazek announced that they had observed an increase in the number of inclusions in the epithelial cells, which they had attempted to cultivate.

A fair degree of headway seemed thus to have been made in the solution of the problem of trachoma, when Heymann (48) announced that he had met with similar inclusions in several cases of gonorrheal blenorrhea neonatorum, and expressed the opinion that they represented a specific reaction to the gonococcal virus. A thorough study of this question then led to the interesting discovery of the existence of an inclusion blenorrhea as a malady *sui generis*, which primarily affects the genitalia of both male and female and secondarily the eyes of the new-born. This type of blenorrhea it is now known may be associated with a gonococcal infection, as well as with other bacterial infections (pneumococci, diphtheria bacilli), but when this occurs the processes are independent of each other.

The discovery of the occurrence of inclusions in connection with blenorrhea of this type naturally threw doubt upon the correctness of v. Prowazek's view, that the constituent granules making up the inclusions found in trachoma actually represented the trachomatous virus. Various suggestions have accordingly been made to account for their appearance in trachoma, on the one hand, and in inclusion blenorrhea, on the other. Lindner (49) did not hesitate to declare the two processes as identical, for in two baboons which he inoculated with pure inclusion blenorrheal material he claims to have obtained a clinical and pathological picture which could not be distinguished from trachoma. Wolfrum (50) even went so far as to inoculate the secretion

from a pure case of inclusion blenorrhea neonatorum into a normal eye of a human being, with the consequent development of the typical picture of trachoma. These two experiments would seem to settle the question of the identity of the virus of trachoma and inclusion blenorrhea were it not for the fact that ophthalmologists do not seem to agree in their opinion whether the diagnosis of trachoma can be made with certainty during the early stages of the malady, i.e., at a time when pannus and scar tissue formation have not yet developed, and when granules represent the sole lesion.

One point which has struck the writer as rather peculiar is that inclusions in trachoma supposedly can only be demonstrated during the very earliest stages of the disease, and that later on they are absent, the inference being that when they cannot be found the condition must be viewed as having passed beyond the earliest stage. This, however, is not always true, and one asks oneself whether Heymann may not be correct in assuming that trachoma is after all a disease *sui generis*, the cause of which is still unknown, and that those cases, where inclusions are demonstrable, represent a double infection, viz., of trachoma with inclusion blenorrhea superadded, and that the inclusions really do not belong to the picture of trachoma. A great deal of work is evidently yet to be done before this problem is solved, and it is rather surprising that during the past ten years so few investigations in this direction have been undertaken. If the enormous importance of trachoma as a public health problem and a source of human misery in so many parts of the world be borne in mind, this should constitute a sufficient impetus for prompt and active investigation, aside from its interesting scientific aspects.

e. Filterable viruses and plant and insect diseases. In conclusion we would briefly refer to the growing interest which the filterable viruses have elicited amongst plant pathologists and entomologists.

It will be recalled that the existence of the filterable viruses was first discovered in a plant disease, viz., the so-called mosaic disease of tobacco, the economic importance of which is sufficiently attested by the fact that the United States Department of Agriculture in its Bureau of Plant Industry maintains a special division in which the etiology and measures of control of this disease are continually being studied. Doctor Allard's name deserves special attention in this connection, for he has unquestionably done more than any other investigator in elucidating the nature and properties of the virus and its mode of dissemination (51).

The demonstration of the connection of a filterable virus with the mosaic disease of tobacco has naturally led to a study of other diseases which affect plants, that are of interest to the agriculturalist, such as the mosaic disease of cabbage, of corn, of Irish potatoes, of cucumbers, of beans, of sugar corn and other grasses from a like angle. The initial studies which have thus far been made clearly show the enormity of the problem.

It has been shown already that in the dissemination of these diseases in the plant world insects probably play a very important rôle, and an investigation of the viruses within the insects has thus suggested itself as yet another field for the study of these interesting disease-producing agencies.

That insects in turn may not merely act as transmitters of filterable virus diseases to plants and animals (e.g., Pappataci fever, dengue, catarrhal fever of sheep, etc.), but may themselves fall a prey to their activity, has been established in several instances, viz., in connection with the so-called polyhedral disease (52) (jaundice) of silk worms (v. Prowazek), the wilt disease of the European nun moth (53) (Wahl), and the American gypsy moth caterpillars (R. Glaser) (54) and the sacbrood of bees (White) (55). So far as we are acquainted with the filterable viruses, they are destructive or at least harmful to their hosts, but that some of them may also prove of benefit, to men at least, is indicated by the fact that the appearance of wilt disease amongst gypsy moths has probably done more to bring about the eradication of this pest in this country than all attempts at control by man.

BIBLIOGRAPHY

(1) Iwanowski, D. 1892. Ueber zwei Krankheiten der Tabakspflanze. (Abst). In Beiheft Botan. Centbl., iii, 1893, 266. Original in Land- u. Forstwiss. Russian; see also Bull. acad. imper. d. sci. de St. Petersburg, xiii, 237.

(2) Beijerinck, M. W. 1899. Ueber ein Kontagium vivum fluidum als Ursache der Fleckenkrankheit der Tabaksblaetter. Centbl. f. Bakt., O., v, 27.

(3) Loeffler and Frosch. 1898. Berichte der Kommission zur Erforschung der Maul- und Klauenseuche, etc. Centbl. f. Bakt., O., v.

(4) Lipschuetz, B. 1913 Filtrierbare Infektionserreger. Kolle and Wassermann's Handbuch der pathogenen Mikroörganismen, viii.

(5) Giemsa G. and v. Prowazek, S. 1908. Weitere Untersuchungen ueber sogenannte ultramikroskopische Infektionserreger: Zur Filtration des Huehnerpestvirus. Muench. med. Wochenschr., no. 29.

(6) ANDRIEWSKY, P. 1915. L'ultrafiltration et les microbes invisibles. Centbl. f. Bakt., O., lxxv, 90.

(7) DUGGAR, B. M. AND J. L. KARRER. 1921. The size of the infective particles in the mosaic disease of tobacco. Ann. Missouri Botan. Gardens, viii.

(8) v. PROWAZEK. S., 1910. Weitere Untersuchungen ueber das Vaccinevirus. Centbl. f. Bakt., O. lvi.

(9) REMLINGER AND LANDSTEINER. 1905. Action de la centrifuge sur le virus rabique. Compt. rend. soc. Biol.

(10) MACCALLUM, W. G. AND E. H. OPPENHEIMER. 1922. Differential centrifugation. A method for the study of filterable viruses, as applied to vaccinia. Journ. Amer. Med. Assoc., lxxviii, no. 6.

(11) BAUER, E. 1904. Zur Aetiologie der infektiösen Panachierung. Ber. d. Deutsch. Bot. Gesell., xxii, 453.

1906. Ibid., xxiv, 416.

1907. Ibid., xxv, 410.

1908. Ibid., xxvi, 711.

(12) D'HERELLE, F. 1917. Sur un microbe invisible antagoniste des bacilles dysentériques. Compt. rend. Acad. sci., clxv, 373. For details regarding this question see also:

1922. The bacteriophage. Transl. by George H. Smith. Baltimore, Md.

(13) DOERR, R. 1921. Die Bakteriophagen. Klin. Woch., I, no. 30 and 31.

(14) BORDET, J. AND CIUCA, M. 1920. Le bacteriophage de d'Herelle. Compt. rend. Soc. Biol., lxxxiii, 1296.

GRATIA, A. 1921. Studies on the d'Herelle phenomenon. Journ. Exper. Med., xxxiv, 115.

BAIL, O. 1921. Das bakteriophage Virus von d'Herelle. Wien. Klin. Wochenschr., xxxiv, 237.

(15) REICH, W. W. AND T. D. BECKWITH. 1922. Trypanosoma brucei as a filterable virus. Journ. Parasit., ix, 93.

(16) REED, CARROLL, AGRAMONTE AND LAZEAR. 1900. Preliminary note on the etiology of yellow fever. Philadelphia Med. Journ., October 27th.

(17) OLITSKY, P. K. AND L. GATES. 1922. Experimental studies of the nasopharyngeal secretions from influenza patients. VIII. Further observations on the cultural and morphological characters of Bacterium pneumosintes. Journ. Exper. Med., xxxv, no. 6.

(18) BORREL. 1903 Étude expérmentale de la clavelée. Annal. de l'Inst. Pasteur.

(19) v. PROWAEZK, S. 1907. Chlamydozoa. Arch. f. Protistenkunde, x.

(20) LIPSCHUETZ, B. 1908. Ueber mikroskopisch sichtbare, filtrierbare Virusarten (Ueber Strongyloplasmen). Centbl. f. Bakt., O. xlviii.

(21) LIPSCHUETZ, B. 1921. Untersuchungen ueber die Aetiologie der Krankheiten der Herpesgruppe Arch. f. Dermat. u. Syph., cxxvi, 428.

(22) LIPSCHUETZ, B. 1922. Untersuchungen ueber Paravaczine. Arch. f. Dermat. u. Syphilis, cxxvii, 193.

(23) DA ROCHA LIMA. 1913. Zur Histologie der Verruga peruviana. Centbl. f. allgem. Pathol. u. pathol. Anat. Verhandl. d. deutsch. path. Gesellsch. zu Marburg., xxiv, 409.

(24) Gins, H. A. 1922. Mikroskopische Befunde bei experimenteller Maul und Klauenseuche. Centbl. Bakt, O. lxxxviii, 265.

(25) Grueter. 1913. Cited by Loewenstein. Wien. Klin. Wochenschr., xxxii, 1919, 952.

(26) Loewenstein. 1919. Das Virus des fieberhaften Herpes. Wien. Klin. Wochenschr., xxxii, 952. Aetiologische Untersuchungen ueber den fieberhaften Herpes. Muench. med. Wochenschr., lxi, 769.

1920. Das Virus des fieberhaften Herpes. Berlin. Klin. Wochenschr., lvi, 1222 Uebertragungsversuche mit dem Virus des fieberhaften Herpes. Klin. Monatsbl. f. Augenheilk., lxiv, 15.

(27) Luger and Lauda. 1921. Zur Kenntniss der Uebertragbarkeit der Keratitis herpetica des Menschen auf die Kaninchenkornea. Wien. Klin. Wochenshr., xxxiv, 132.

Zur Aetiologie des Herpes febrilis. Ibid., xxxiv, 251, and Zeitsch. f. d. gesammt. Exper. Med., xxiv, 289.

(28) Blanc and Caminopetros. 1921. Recherches expérimentales sur l'herpès. Compt rend Soc Biol, lxxxiv, 629, 767, 859. Ibid., lxxxv, 290.

(29) Levaditi, C. and P. Harvier. 1920. Étude expérimentale de l'encéphalite dite léthargique. Annal. Inst. Pasteur., xxxiv, 911.

Levaditi, C., P. Harvier and S. Nicolau. 1922. Ibid., xxxvi, 63.

(30) Doerr, R. and A. Schnabel. 1921. Das Virus des Herpes febrilis und seine Beziehungen zum Virus der Encephalitis epidemica. Zeitschr. f. d. ges. Hygiene u. Infekt., xciv, 29.

Schnabel, A. 1923. Die Aetiologie der Encephalitis epidemica. Klin. Wochenschr., ii, 429.

(31) Strauss, I., S. Hirschfeld and L. Loewe. 1919. Studies in epidemic encephalitis. Journ. Inf. Dis., xxv, 378.

Loewe, L. and S. Strauss. 1920. Studies in epidemic encephalitis. Ibid., xxvii, 250. Experimental studies in encephalitis lethargica. Proc. New York Path. Soc., xx, 18.

(32) Kling. 1921. Cited by R. Doerr and S. Zdansky. In "Zur Aetiologie der Encephalitis epidemica." Schweiz. med. Wochenschr, 1922, liii, 349, from Hygiea, 1921, Heft 21 and Compt. rend. Soc. Biol., lxxxvii, 1179.

(33) Olitsky, P. K. and F.L.Gates. 1920. Experimental studies of the naso-pharyngeal secretions from influenza patients. I. Transmission experiments with naso-pharyngeal washings. Journ. Exper. Med., xxxiii, 125.

1921. II. Filterability and resistance to glycerol. Ibid., 361. IV. Anaerobic cultivation. Ibid., 713.

1922. VIII. Observations on the cultural and morphological characters of Bacterium pneumosintes. Ibid., xxxv, 813.

Investigations on the bacteriology of epidemic influenza. Science, lvii, 159.

(34) Hiraishi and Okamoto. 1921. Japanese Med. World, i, 10. Cited by Marie, P. L. La Presse médicale. 1922. Aug. 19.

Kawamura. 1922. Ibid., ii, 31. Cited by Marie.

(35) Goldberger, J. and J. F. Anderson. 1911. The nature of the virus of measles. Journ. Amer. Med. Assoc., lvii, 971.

(36) NICOLLE AND CONSEIL. 1921. Compt. rend. Soc. Biol., lxxxiii, 56.
1921. Compt. rend. Acad. Sci., cliii, 1522.
(37) HEKTOEN, L. 1905. Experimental measles. Journ. Inf. Dis., ii, 238.
(38) SELLARDS, A. W. 1919. Insusceptibility of man to inoculation with blood from measles patients. Ibid., xxx, 257.
(39) BLAKE, F. G. AND J. D. TRASK, JR. 1921. Studies on measles. I. Susceptibility of monkeys to the virus of measles. Journ. Exper. Med., xxxiii, 385.
(40) NEVIN, M. AND F. R. BITTMAN. 1921. Experimental measles in rabbits and monkeys. Journ. Infect. Dis., xxix, 429.
(41) DUVAL, C. W. AND R. d'AUNOY. 1922. Studies upon experimental measles. I. The effect of the virus of measles upon the guinea pig. Journ. Exper. Med., xxxv, 257. II. The enanthematous, exanthematous, pyrexial and leucocytic syndrome produced in the rabbit by intravenous inoculation of blood. Ibid., xxxvi, 231. III. The symptom-complex in the guinea pig and rabbit following the intratracheal and intravenous injection of filtered naso-pharyngeal secretions from cases of human measles. Ibid., xxxvi, 239.
(42) GRUND, M. 1922. Susceptibility of rabbits to the virus of measles. Inoculations with naso-pharyngeal material. Journ. Infect. Dis., xxx, 86.
(43) TUNNICLIFF, R. AND W. B. MOODY. 1922. Experimental measles by inoculation of monkeys, guinea pigs and rabbits with a green producing diplococcus. Journ. Infect. Dis., xxxi, 382.
(44) HALBERSTAEDTER AND S. v. PROWAZEK. 1907. Ueber Zelleinschluesse parasitaerer Natur bei Trachoma. Arbeiten aus dem Kaiserl. Gesundheitsamt., xxxvi, H. 1.
1909. Ueber Chlamydozoenbefunde bei Blennorrhoea neonatorum non-gonorrhoeica. Berl. Klin. Wochenschr., no. 24 and 41.
(45) BERTARELLI AND CECHETTO. 1913. Die Filtrierbarkeit des trachomatoesen Virus. Centbl. Bakt., O. lxx.
(46) NICOLLE, CUÉNOD AND BLAIZOT. 1912. Le Magot, animal reactif du trachome. Filtrabilité du virus. Pouvoir infectant des larmes. 1913. Compt. rend. Acad. Sc., 1912, 241; 1913, 1177.
(47) NOGUCHI, H. AND COHEN. 1913. Experiments on the cultivation of so-called trachoma bodies. Journ. Exper. Med., xviii.
(48) HEYMANN, B. 1909 Ueber die Trachomkoerperchen. Deutsch. med. Wochenschr., xxxv, 1692.
(49) LINDNER, K. 1909. Uebertragungsversuche von gonokokkenfreier Blennorrhoea neonatorum auf Affen. Wien. Klin. Wochenschr., 1555 and 1659.
1913. Zur Biologie des Einschlussblennorrhoea-(Trachom-) Virus. v. Graefe's Arch. f. Ophthal., lxxxiv.
(50) WOLFRUM. 1910. Ueber die Einschlusserkrankungen der menschlichen Bindehaut. 36te Versammlung d. ophthal. Ges. Heidelberg, p. 207.
(51) ALLARD, H. A. 1914. The mosaic disease of tobacco. U. S. Dept. Agric. Bull. 40.
1916. Some properties of the virus of the mosaic disease of tobacco. Journ. Agric. Research., vi, 649.
1917. Further studies of the mosaic disease of tobacco. Ibid., x, 615.

(52) v. Prowazek, S. 1907. Chlamydozoa. II. Gelbsucht der Seidenraupen. Arch. f. Protistenk., x, 359.
(53) Wahl, B. 1909. Ueber die Polyederkrankheit der Nonne. Centbl. Gesam. Forstwiss. 1912, xxxv, 164, 212, xxxvi, 193, 377; xxxvii, 247; xxxviii, 355.
(54) Glaser, R. W. 1915. Wilt of gypsy-moth caterpillars. Journ. Agric. Research, iv, 101.
(55) White, G. F. 1917. Sacbrood. U. S. Dept. Agric. Bull., 431.

MINERAL METABOLISM IN RELATION TO ACID-BASE EQUILIBRIUM

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Liebig's researches in 1840 (125), (126) on the minerals in plants and in animal tissues were the first scientific approach to the problem of mineral metabolism. Then in rapid succession came the analyses of bone by Bibra, in 1844 (9); the discovery of the alkaline tide of the urine by Bence Jones in 1850 (105); studies on the effects of acids and alkalies on mineral excretion by Buchheim and his students at Dorpat, (29), (41), (150), (236) in 1850–1865; and the researches of Bunge (25), (26) who worked more than twenty years on the composition of blood and milk and the action of salts. Soon the problems of mineral metabolism spread to many fields. Reviews of the early literature have been made by Voit in 1882 (227), and Loewi in 1907 (129), (130). In 1906 Albu and Neuberg (3) published the first monograph on the subject. A monograph by Forbes and Keith (49) contains references to all the aspects of metabolism studies in which phosphorus plays a part. Matill and Matill (140) have made the latest review of mineral metabolism.

In recent years investigations emphasize the physiological importance not only of minerals but also of the resultant hydrogen ion concentration to botany and soil chemistry, to muscle contraction, nerve irritability, cell permeability and bacterial growth. In metabolism the new type of biological experiments—feeding animals with purified substances—necessitated the careful consideration of salts in food. New studies have also clarified problems in acidosis and alkalosis. Data obtained from these various sources have renewed interest in mineral metabolism of human beings.

Most studies have been fragmentary—each investigation has dealt with one or several mineral constituents. A few complete studies were made, but even from these no conclusions have been drawn as to the significance of mineral metabolism in acid-base economy. In order to recognize this relationship, intake and output of salts must

be calculated in common terms; namely, as normal solutions. The analogy to energy metabolism is obvious. Salts can be evaluated in cubic centimeters of normal solution just as grams of protein, carbohydrate and fat can be reckoned in calories. The calcium requirement can be expressed in cubic centimeters of normal solution or per cent of the total cubic centimeters of salt, just as the protein requirement can be stated in calories or in per cent of the total calories. Given the total requirement, the extent to which salts are interchangeable must be determined for the various radicals, just as isodynamic quantities of fat and carbohydrate can be calculated. Amounts of minerals can be compared directly as chemical equivalents. It can thus be determined whether food and excreta are acid or basic and whether the metabolism has resulted in a gain or loss of acids or base.

Mineral metabolism studies must be recalculated in terms of acid or base. A measurement of intake and output of a substance shows the "balance." When acid or base is retained, the balance is positive; when excreted in excess of intake, the balance is negative; and when the body neither gains nor loses, it is said to be in acid-base equilibrium. The value of acid or basic solutions can be stated in cubic centimeters of normal solutions—plus sign for acid or minus sign for base. For purposes of the computation of analyses in terms of acid or base, sodium, potassium, calcium and magnesium form the "base" value; sulphur, chlorine and phosphorus, the "acid" value. Sodium, potassium and chlorine are monovalent; calcium, magnesium and sulphate are divalent. Phosphorus is calculated by Sherman as divalent, but in the body it is 1.8. The algebraic sum gives the excess of acid or base. Such problems, as the fractions of the phosphorus and sulphur which are burned to form acid radicals, make accurate computation difficult. In stating the value of acid-base metabolism, it is convenient to speak not of acid balance, but always of "base balance." The acid or base which is lost or retained gives rise to four possibilities.

1. Base retention = positive base balance
2. Acid excretion in excess of intake = positive base balance
3. Base excretion in excess of intake = negative base balance
4. Acid retention = negative base balance

From the many excellent metabolism studies, those have been selected in which all the acid and basic elements have been determined in food, urine and feces and which, therefore, permit critical analysis of acid-base economy. Elements which have not an important acid or base value—as iron, iodine, fluorine, manganese, arsenic, silicon, etc.—will

not be covered in this paper. Though they may be essential for life processes, they occur in such minute amounts that for metabolic experiments their acid or base value can be neglected. We shall discuss mineral metabolism from five main viewpoints: *1*, acid-base requirements; *2*, acid-base metabolism; *3*, acid-base equilibrium in the body; *4*, the effect of acid on mineral metabolism; *5*, the effect of alkali on mineral metabolism.

ACID-BASE REQUIREMENTS. That the body requires minerals for carrying on its functions was clearly stated by Bence Jones in 1850, (105, p. 3):

> Some idea of the comparative value of different classes of substances is obtained by noting the rapidity with which animals die when any one of them is absent from their food. Thus, the following order might be formed, 1st, air; 2nd, water; 3rd, organic substances containing nitrogen; 4th, organic substances free from nitrogen; 5th, inorganic substances, the ashes, salts.

Further, (p. 7):

> With white of egg . . . . the animal fed on it alone died of starvation. The same results follow from a diet of sugar or fat alone; and, if nitrogenous and non-nitrogenous food was given perfectly free from all saline matter, perfect nourishment of the body could not take place. Still more than this, if one single salt which exists in the body was left entirely out of the food, disease, if not death, results.

Salt starvation. The above statements are clear but lack convincing experimental proof; they were given weight by the classic experiments of Forster (52). He showed that diets which were salt free but otherwise adequate caused death.

> Pigeons, which were fed with salt poor diets, died in thirteen, fifteen, and twenty-nine days; two dogs likewise fed, after twenty-six and thirty-six days, were near death. Because the animals were in nitrogen equilibrium, death can only be attributed to the lack of the ash materials in the diet. The intake of these should not fall below definite limits. With the most complete removal of the minerals in the diet of grown animals, the processes of metabolism, catabolism, and destruction go on in the same way as in the usual diet, adequate in all respects. Still profound disturbances in the function of the organs come about, which hinder changes of the food material into absorbable modifications and the substitution of destroyed body materials. In part the processes necessary for life are depressed before the impossibility of a lasting food absorption draws destruction and death after it.
>
> Although the dog remained in approximate nitrogen equilibrium on a diet of meat powder, fat, and starch, the salt content of excretion was small but was always greater than the intake. Stupidity, loss of interest, trembling, muscle weakness, paralysis of the hind legs and later convulsions and raging developed.

Although the body contained probably 1500 gm. of salts at the onset, this condition entailed the loss in feces and urine of only 30 gm. P_2O_5 and 7 gm. NaCl in twenty-six days.

Bunge felt this might be due to the production of sulphuric acid in the metabolism of the protein. At his suggestion, therefore, Lunin (132) repeated these experiments on mice, using a diet containing only 0.06 per cent of ash. To this he added $NaHCO_3$. The animals receiving the alkali lived longer than the controls, but died showing similar symptoms.

Taylor (220) repeated these experiments on himself. He took a diet consisting of 70 grams of washed coagulated egg white, 120 grams of washed fat and 200 grams sugar—comprising 2250 calories—and 1 gram ash. There was nitrogen equilibrium; the sulphur in the urine was fairly constant and of such magnitude as to indicate that it came from the food. Phosphate excretion reached a constant level of 0.8 gram PO_4, chlorine fell to 0.2 gram per day, and calcium and magnesium disappeared from the urine after the fourth day. The acidity of the urine was measured by the hydrogen electrode but the value is probably incorrect. The urine is too acid for the accompanying ammonia, which stayed constant. On the ninth day acetone was observed on the breath and the experiment was discontinued. Taylor describes his main complaint as loss of appetite; his nervous symptoms were soreness in the muscles, with normal reflexes and circulation. There was marked diaphoresis and diuresis. He lost 1.5 kgm. of weight but this was regained as soon as the experiment was discontinued. Since the feces were not analyzed, it is impossible to compute the balances.

Goodall and Joslin (70), working under Folin's direction, repeated this experiment on two students, giving attention to the fractions of nitrogen and sulphur. They kept one man on the diet for thirteen days and the other for nine days. They confirmed Taylor's experiments in all particulars, except that no acetone was formed. They also failed to analyze the feces, so that no data are available as to the minerals lost in metabolism. They also bring out the fact that there was rapid loss of weight. One man lost 2.5 kgm. in the first three days and 2.5 kgm. in the next ten days. In the seventy-two hours after the experiment was discontinued he gained 4.1 kgm.

V. Wendt (234) has studied his complete mineral metabolism during fourteen days for 1st, a diet low in both ash and protein; and during twelve days for 2nd, a diet low in ash and liberal in protein. These are not reported in detail because he made a series of salt additions

which, therefore, make interpretation difficult. To calculate the base balance is also difficult since he does not give the separate values for sodium and potassium.

This remarkable group of experiments should be repeated and the total mineral metabolism determined. The conditions in salt starvation differ from those in fasting; in salt starvation no body protein is destroyed, with consequent acid production. Hence the acid-base value of the excretion should be different.

Fasting. Benedict (8) determined all the acids and bases in the urine of a fasting man over the whole period of thirty-one days and, as there were no stools, the values represent the total excretion. Com-

TABLE 1

Benedict's fasting man—Average excretion per day

	cc. 0.1 N		cc. 0.1 N
Cl	111	Ca	105
P	465	Mg	55
S	355	K	228
		Na	114
(1) Acids	+931		
(2) Bases	−502		−502
(3) = (1) − (2) Excretion	+428		

puted on the basis of normal solutions, as shown in table 1, the excretion represents a daily average for thirty-one days of 931 cc. 0.1N of acids radicals, 502 cc. of alkali radicals, or an excretion of acid of 428 cc. 0.1N. For the total period, 13,268 cc. 0.1N acid were excreted from an alkaline blood.

In fasting the great loss in weight is largely due to the loss of fluids. There is also a breakdown of body substance. Mineral excretion represents the sum of these two. Gamble (1921) has taken into consideration this loss of "body water" as a source of mineral output. The data, when published, will permit calculation of the acid base metabolism. The loss of body fluids in salt starvation and fasting indicates the practical importance of acid-base metabolism in anhydremia.

Normal requirements for adults. The need of minerals can be ascertained from dietary studies of freely chosen dietaries. The first study of this type was that of Sherman on iron (197). Shortly following appeared that of Sherman, Mettler and Sinclair (198), of

Tiegerstedt (221), and of Horneman (91) on calcium, magnesium and phosphorus.

Sherman has completed his original purpose of analyzing diets for all the elements and has given the average intakes of the mineral constituents of 150 American dietaries (199, p. 271) calculated on per man per day and per 3000 calorie basis. The minimum is probably something below, and the average above, the actual requirement. These can be compared with data he has more recently published, derived from metabolism experiments (204); the calcium requirement, average of 97 studies, is 0.45 gram, or 225 cc. per man per day (201) and the

TABLE 2

Sherman's data for minerals in food, calculated as normal solutions

ACIDS			ALKALI		
	cc. 0.1 N			cc. 0.1 N	
	Minimum	Average		Minimum	Average
P	418	925	Ca	175	365
Cl	233	810	Mg	140	283
S	498	813	K	418	870
			Na	96	850
(1) Acids	+949	+2548			
(2) Bases	−829	−2368	Bases	−829	−2368
(3) = (1) − (2) Food	+120	+180			

phosphorus requirement, average of 95 studies, is 0.88 gram, or 464 cc. 0.1N. Sherman's figures in terms of acid or base value per 3000 calories per day are recalculated in table 2.

Blatherwick (17) has by similar calculations found in thirty-two army dietary studies an average of 22 cc. 0.1N acid per man per day, with variations from 494 cc. of acid to 250 cc. of alkali. Since these diets were adequate in other respects and the men thrived upon them, it is only fair to assume that other freely chosen mixed diets would vary as widely in acid-base values when studied over short periods. Because Sherman's data are so well checked by metabolism studies, they more accurately represent the needs of the body. The normal requirements of an adult are, therefore, approximately 150 cc. 0.1N acid per day.

ACID-BASE METABOLISM. Dietetic studies on large groups to determine the normal consumption of minerals give us no insight as to

their use in the body. The changes which take place can be determined only roughly. Inadequacy of the diet is shown by gross pathological changes or death. The acid-base metabolism can be calculated from suitable data in the literature for *1*, fetus; *2*, infant; *3*, child; *4*, adult; *5*, women during pregnancy and lactation.

Fetus. For the fetus the problem of mineral metabolism is simplified. We cannot estimate the intake and output, but can only take into account the balance. The balance is, of course, positive for all elements and represents the retention of minerals during growth. Extensive work has been done on ash analysis of the fetus and the newborn. There is an excellent review of this material in Czerny and Keller (32), chapter 14. The original object of much of the work was to determine whether Bunge's theory that the ash of the fetus resembled the mother's milk rather than the mother's blood could be substantiated. The facts did not bear out this theory—and Bunge was led to modify the theory to the following—that the time required for the fetus to double its weight was dependent upon the composition of the mother's milk. However, much valuable material has been collected.

The data show the relation of the growth of the fetus to its mineral metabolism. The weight and ash analysis of the fetus are given by Fehling (44) and Camerer (27). The percentage of ash increases from 0.001 per cent at six weeks to 3.3 per cent of total weight at term. As the measurements and weights of the fetus are given, the total ash can be calculated and equals at four months 0.369 gram; at five months, 1.33 gram, at six months, 6.0 grams; at seven months, 26.6 grams; and at term, 100 grams. The percentage of ash and the total value of ash before four months is so small as to be negligible in the maternal metabolism. There is an actual gain in the weight of ash from the fourth to the fifth month of 1.0 gram, from the fifth to the sixth of 5.6 grams, from the sixth to the seventh of 20.0 grams, and from the seventh to the ninth of 73.0 grams. The composition of the ash of fetuses by Hugounencq and Michel, of the new born by Camerer (27) and Söldner (206), and the premature baby by Langstein and Edelstein (121) differ so little that they fall between the limits of analytical errors. The mineral analyses of Camerer and Söldner are given in table 3, and are also calculated as normal solutions. The total ash value of the fetus is 11,970 cc. 0.1N of acid radicals, 18,680 cc. of basic radicals, a result of 6710 cc. of base. The mother must, therefore, supply to the fetus in basic radicals from the fourth to the fifth month

186 cc., from the fifth to the sixth month 1000 cc., from the sixth to the seventh month 3736 cc., and in the last two months 13,600 cc. Stated in other terms, for the last two months the mother must provide daily 230 cc. of basic radicals, 145 cc. 0.1N of acid radicals, resulting in 85 cc. of base. For practical purposes it is sufficient to consider that for the last one hundred days of pregnancy the mother must supply to the fetus salts in the proportions shown in the table, amounting to 1.0 gram of ash per day. This does not take into account the needs for the placenta or the uterus and hence should be considered the minimum of additional salts required for the fetus alone. The value of base removed for the fetus, 85 cc. 0.1N per day, is sufficient to be

TABLE 3

Retention of mineral salts by fetus

Per gram of ash

	GM.	CC. 0.1 N		GM.	CC. 0.1 N
Ca	0.279	140.0	P	0.164	96.0
Mg	0.0086	7.2	S	0.008	5.0
Na	0.057	24.8	Cl	0.0661	18.7
K	0.058	14.8			
			Acids (2)		+119.7
Bases (1)		−186.8			
Acids (2)		+119.7			
Balance (3) = (1) − (2)		−67.1			

considered a cause for the acidosis shown by Hasselbach to exist in late pregnancy. As a minimum, therefore, the diet of the woman during the last three months of pregnancy should contain an addition of 150 cc. 0.1 N base per day.

Infant. The fetus was shown to retain considerable amounts of minerals. So, too, must the infant show retention of minerals during its growth. In a study of its metabolism, intake and output must also be considered. As a result of the interest aroused by the Breslau School, numerous papers have appeared, reviews of which are found in Meyer and Schloss. But in one particular or another they are incomplete and therefore unsuited to the present discussion. Only five cases on normal infants contain data sufficiently complete to permit discussion of the acid-base relationship—of these, two are cases on breast feeding and three on artificial feeding. Blauberg (20), (21) was the first to study the complete mineral metabolism. He

studied two babies, one breast fed and one artificially fed—during the same period in which Rubner and Heubner (176) studied the latter's energy metabolism. Therefore, the data are especially valuable since they permit correlation with other aspects of metabolism.

Breast feeding. The breast-fed baby studied by Blauberg (21) was four and three-quarter months old, weighed 6740 grams and gained 10 grams daily for the six days observed. The values of the minerals calculated in normal solutions, table 4, show a retention of 8 cc. of base per kgm. per day.

TABLE 4

Base balance of infants

Type of feeding	Breast fed	Breast fed	Cow's milk	Cow's milk	Cow's milk
Author	Blauberg	Tobler and Noll	Blauberg	Shohl and Sato	Shohl and Sato
	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N	cc. 0.1 N
Feces value (1) bases	−54	−70	−545	−477	−332
Feces value (2) acids	+14	+18	+261	+123	+ 90
(1) − (2) = (3) total	−40	−52	−284	−354	−242
(4) bases	−66	−49	−376	−357	−289
Urine value (5) acids	+40	+33	+368	+570	+366
(4) − (5) = (6) total	−26	−16	− 8	+213	+ 77
Total output (7) = (6) + (3)	− 66	− 68	−292	−141	−165
Food value (8)	−118	−147	−613	−255	−211
Positive base balance (9) = (8) − (7)	− 52	− 79	−321*	−114	− 46
Positive base balance per kilo	8	18.2	26	12	8

*When 199 cc. 0.1 N are subtracted for negative balances, the value is −222 0.1 N.

Similarly, the baby studied by Tobler and Noll (224) was two and one-half months old, weighed 4 kgm. and gained 25 grams daily for the six days observed. The positive base balance amounted to 18.2 cc. 0.1 N. This is larger than for the baby studied by Blauberg, but the gain in weight was proportionally greater. Also, even though the baby was smaller, he took more food.

Artificially-fed. In Blauberg's second study (20) the baby was seven and one-half months old, weighed 7570 grams, gained daily 21.66 grams for the six days observed, and was fed cow's milk and added lactose. Unfortunately for the value of this experiment, there were

negative balances of chlorine, sulphur and sodium. The baby should show gains for all the minerals. We have in table 4 made a correction for the negative balances, but have not estimated the probable retentions. The value of 26 cc. retention per kilo would then probably more nearly equal the balances of the following two cases.

In the two cases of Shohl and Sato (205), the babies were seven and one-half and nine months old, weighed 8.7 kgm. and 5.5 kgm., gained daily 15 grams each for the three days observed. They were fed on cow's milk and cane sugar. They showed a positive base balance of 12 cc. and 8 cc. 0.1N. Therefore, the metabolism in infancy results in a base retention per kilo per day of 10 to 15 cc. 0.1N.

Child. The data for infant metabolism are voluminous—that for children is rare. Herbst's excellent study (84) is lacking only in the sulphur balance. Schwarz has supplied data on the sulphur metabolism and its relation to the nitrogen so that these values may, for orientation purposes, be substituted. However, we prefer to consider the only complete mineral metabolism study of children available, that of Sawyer, Bauman and Stevens (184). They have studied the metabolism of two children, ages five and eight years, weighing 22.5 and 23.0 kgm., for three-day periods.

We have recalculated their data, not considering ammonia as an alkali, and also calculated the value of the food which they analyzed. Their figures, as shown in table 5, resolve into a retention of 10.0 cc. and 14.5 cc. 0.1N base per kilo per day. The values are practically the same as those found for infants.

The value of retention can be evaluated, as for the fetus, from the composition of the ash of the body. Such figures for normals are more difficult to obtain in children and adults and more data are urgently needed. Brubacher was unable to obtain complete autopsies. The outstanding conclusion from analyses (36), (207), (216) is that the composition of the body of a child, in respect to its minerals, is very similar to that of the fetus and new-born, and is constant in health and disease. As in the fetus, the ash increases at a more rapid rate than does the total weight. If we calculate the ash as 3.3 per cent of the weight at birth and 4 per cent at the end of the first year and the weight at birth as 3.3 kgm. and at the end of the first year as 10.0 kgm., then the ash increases from 100 grams to 400 grams. The total alkali value of the body as calculated from table 2 is 400 × 67.1, or 26,840 cc. 0.1N. This represents the total alkali store of the body at one year. The total increase from birth is 20,100 cc. 0.1N, which equals 58 cc. per day.

Dividing this by the average weight of 6.6 kgm., there is found to be a retention of 9 cc. 0.1N base per kilo per day. This value, obtained by calculation, shows close agreement with the value from metabolism studies of 10 to 15 cc. 0.1N cc. per kilo per day.

Distribution of retained base. What becomes of the base which is stored? Is it possible to account for a major portion? Obviously, as the body grows its content of alkali increases. Among the substances which might neutralize base the first which will be considered is body protein. If a baby weighs 3300 grams at birth and triples its weight in a year, it grows at the rate of about 20 grams a day. If we assume 15 per cent of the body weight as protein, then it gains 3 grams of protein a day. If the molecular weight of protein is 15,000 and it is considered a monobasic salt, then to form an alkali protein salt would require a base $\frac{3}{15{,}000}$ liters normal, or 2 cc. 0.1N alkali per day.

If we calculate the "alkaline reserve" in the manner of Palmer and Van Slyke, assuming the body is 70 per cent fluid and this is 0.03 N $NaHCO_3$, then the baby grows $\frac{6600 \times 0.03 \times 0.70}{365}$ equals 4 cc. 0.1N alkali per day.

The value of the bones must be taken into account since they are known to be largely alkaline phosphate and carbonate, since in infancy the largest deposition of bone growth takes place, and since the bone becomes increasingly rich in carbonate with advancing age. The bone ash at birth can be taken as approximately five-sixths of 100 grams, or 83 grams, and at one year as five sixths of 400 grams, or 333 grams, representing a daily gain of approximately 0.7 gram of ash. If we assume that the ash is 20 per cent $CaCO_3$ and 80 per cent $Ca_3(PO_4)_2$, each gram of $CaCO_3$ would require 200 cc. base and each gram of $Ca_3(PO_4)_2$ 40 cc. base. Therefore, 0.80×40 equals 32, and 0.20×200 equals 40, and for each gram of bone we have 72 cc., or 50 cc. of alkali for bone growth for one day. Thus we have accounted for 2 cc. protein, 4 cc. alkaline reserve, 50 cc. bone, alkaline requirement 56 cc. per day. This leaves only 2 cc. of alkali to be accounted for, as the value of 58 cc. represents the total requirement.

Fats. Fat in excessive amounts, or in ordinary amounts in certain pathological states, has been shown to produce so-called soap stools in infants and children. Keller (108) demonstrated an increase in ammonia in the urines of such infants. Steinitz (216) showed under similar conditions a negative balance of sodium and potassium. The

excretion of these radicals diminished slightly in the urine, but increased greatly in the stool. He therefore suggested that elimination of soaps caused a loss of bases, which left the body richer in acids. This he called a relative acidosis. Rothberg (174), for calcium, and Birk (12), for magnesium, were able to show that in chronic nutritional disturbances increased fat caused negative balances. Keller (109), Freund (56), Bahrdt (7) and Givens (65) corroborated these findings. Thus very strong evidence has been collected to establish the theory of relative acidosis.

Sawyer, Bauman and Stevens have also studied the effect of fat on mineral metabolism. On the same two children as were studied during normal periods, they determined the balance of all the minerals when fat was substituted in their diet for an equal number of calories of carbohydrate. Acidosis was produced. The alkali reserve which was 50 and 49 vol. per cent on the normal diets was reduced to 29 and 37 vol. per cent. The values in table 5 show that the positive base balance was reduced in C.G. from 10.0 cc. (normal diet) to a negative balance of 0.8 cc. 0.1N (high fat diet) per kilo per day, and in R.W. from 14.5 cc. (normal diet) to 6.0 cc. 0.1N (high fat diet) per kilo per day. During the periods when the children were in a state of acidosis the alkali retention was reduced to half the normal or eliminated.

The justification of Steinitz's theory of the action of fat is not tenable upon the finding of negative balances. The acidosis is only in part caused by loss of bases in the feces. It depends also upon the failure of the urine to secrete acid. Ammonia excretion decreased in one case of Sawyer, Bauman and Stevens. Therefore, ammonia is not an essential feature of the acidosis, but only occurs when organic acids are also excreted in the urine.

The mechanism by which the acidosis developed can be seen by studying the paths of excretion, table 5. The food value for three days for the normal periods contained 363 cc. less acid than in the fat periods. The value of the urine for C.G. was +1459 cc. and +1253 cc., and R.W. +1787 cc. and +1698 cc. The value of the stools for C.G. was −343 and −520 and for R.W. −349 and −478. The value of the total excretion for C.G. was +1116 and +733 and for R.W. +1438 and +1220. The acid excretion in the urine was actually reduced in both cases by fat, 206 cc. for C.G. and 99 cc. for R.W. The value of the stools was alkaline and was increased in both cases by fat, 179 cc. for C.G. and 129 cc. for R.W. Therefore, the acid

excretion was diminished both by a less acid urine and a more alkaline stool. The balance is a result of three coöperative factors: increased acid in the food and diminished acid excretion in the urine and feces.

Adults. The only complete study of the mineral metabolism of the adult has been made by v. Wendt (234). He has determined the mineral balance of two subjects—G. and L.—on a normal diet, with varying salt content, for four days. On two days the data are complete. Unfortunately, in the data he did not give the values of sodium

TABLE 5

Sawyer, Bauman and Stevens—Base balance of children

	C.G., NORMAL (cc. 0.1 N)	C.G., HIGH FAT (cc. 0.1 N)	R.W., NORMAL (cc. 0.1 N)	R.W., HIGH FAT (cc. 0.1 N)
Feces value (1) Bases	−905	−1366	−1149	−1135
Feces value (2) Acid	+562	+ 846	+ 800	+ 657
(Difference 1–2) (3) Total	−343	− 520	− 349	− 478
Urine value (4) Bases	−3738	−4482	−3446	−4326
Urine value (5) Acid	+5197	+5735	+5233	+6024
(Difference 5–4) (6) Total	+1459	+1253	+1787	+1698
Total output (difference 6–4) (7)	+1116	+733	+1438	+1220
Food value (difference 7–8 (8)	+ 426	+789	+ 426	+ 789
Base balance for 3 days	pos. 690	neg. 56	pos. 1012	pos. 431
Base balance for 1 day	pos. 230	neg. 17	pos. 334	pos. 134
Base balance per kilo per day	pos. 10.0	neg. 0.8	pos. 14.5	pos. 6.0

and potassium separately and the values assigned to them are approximated by calculating the average ratio of Na/K in the food as 3/1. The data would be more valuable if the subjects were in equilibrium throughout the experiment and studied for longer periods. The food amounts to −27 cc. 0.1N. There are positive base balances for G. of 973 cc. 0.1N and 226 cc. 0.1N. For subject L., similarly, there are positive base balances of 759 cc. 0.1N and 240 cc. 0.1N. per kilo per day; this equals a retention of 13.7 and 3.2 cc. 0.1N for G. and 13.3 and 4.2 cc. 0.1N for L.

Pregnancy and lactation. The requirements of minerals for the adult man may be very different from those of a woman during pregnancy

and lactation. In order to retain her own store of minerals, she must store a surplus for the fetus. No complete studies in mineral metabolism are available for an estimation of the balances in humans. Therefore, Forbes' studies (48) on the metabolism of the milch cow are offered as suggestive. Forbes, with others, has published complete mineral metabolism studies on forty-nine cows, for twenty day periods, throughout the yearly cycle of pregnancy and lactation. Minerals have been added, including various forms of calcium and phosphorus. The data are in such form as to permit calculation of the base balance, as well as the balances of the individual constituents. His results show negative balances, regardless of the mineral content of the diet, during lactation, and lactation combined with pregnancy. Not until lactation has ceased and late in the next pregnancy does the balance of calcium and the other elements become positive.

TABLE 6

Forbes' data on metabolism of the milch cow

WEIGHT	DAILY GAIN	PERIOD	COW	SUPPLE	PERIOD OF LACTATION	PERIOD OF GESTATION	MILK	BASE BALANCE PER KGM. CC. 0.1 N
kg.								
519	7.08	II	3		1–7		47	pos. 2.4
514	1.11	II	10		nearly dry	178–193	10	pos. 5.7
579	0.17	I	4		dry	240–259		neg. 7.0
594	0.50	II	4	Ca.	dry	275–279		pos. 5.8

Only four experiments from the whole series will be considered for the purpose of illustration, as shown in table 6. In three of the four cases there is a positive base balance per kilo per day, amounting to 2.4, 5.7, and 5.8 cc. 0.1N; in one, there is a negative base balance of 7.0 cc. 0.1N. In cow 3, period II, the base balance is positive because of excretion of acids. In cow 4, period I, the base balance is negative because of retention of acids. In the remaining two cows the base balance is positive due to retention of alkali. A base retention during pregnancy and lactation is the result of two causes: Milk secretion causes a negative base balance, and the alkali requirement of the fetus causes a positive base balance. In the pregnant cow the alkali retention is half that of the normal infant.

Partial studies in the mineral metabolism of pregnant women have been reported by Landsberg (118), (119). He has studied the metabolism of nitrogen, phosphorus, sulphur, magnesium and calcium. He

concludes that from the second to the tenth month of pregnancy the mother on very liberal intakes of 16.0 grams N, 3.0 grams = 1740 cc. 0.1N P, 0.5 gram = 402 cc. 0.1N Mg, and 2.5 grams = 1250 cc. 0.1N Ca, shows positive balances of all these elements. Even after deducting the amount necessary for the fetus—which he calculated from data already cited under the fetus—he found large positive balances for the mother. The fetal adnexa and the growing uterus must require their quota, or the mother must be storing minerals upon which to draw during lactation.

No metabolism experiments upon women during lactation are available. We can, however, compute, from the analysis of breast milk, that quota of alkali which she must supply. In Blauberg's case the value of the breast milk consumed was 188 cc. 0.1N alkali per day; in Tobler and Noll's the milk equalled 147 cc. 0.1N alkali per day. Hence, this amount of minerals must be given as a minimum to the nursing mother in addition to her normal requirement. The practical importance of determining what is an adequate mineral diet and the alkali requirement for a pregnant or nursing woman warrants further investigation.

ACID-BASE EQUILIBRIUM IN THE BODY. The acid-base equilibrium of the body is governed by the intake of acids in the food and their excretion in the urine and feces.

Acid-base value of food. The word alkali comes from the Arabic and means marine plants. Liebig speaks of the acid ash of meats, meat juice and cereals and the alkali ash of vegetables. The value of foods as sources of an excess of acid or base forming elements was first expressed in terms of normal solutions by Meyerhofer (147) in 1902 for mineral springs; and by Dennstedt and Rumpf (35) in 1904, for animal tissue. To Sherman we really owe our present knowledge of the subjects. He has given (208), (210) tables of the acid or base value of foods in cubic centimeters of normal solutions per 100 grams and per 100 calories. Forbes (51) published from his own experiments and analyses in the literature a table of calculations of acid-base value of foodstuffs. The most complete data are those of Berg.

The significance of acid and alkaline diets has been reviewed by Greenwald (15, p. 413), who states no relation to metabolism has been proved. Blatherwick has found the diets in army camps to average 22 cc. 0.1N acid. Sherman found an average value of 180 cc. 0.1N acid on freely chosen mixed diets. The civilian diets should approximate the requirements but the army diets should be more satisfactory

because every effort was made to give the soldiers the best diet available. These studies were made over short periods. Even during long periods the acid or alkaline value may not determine life or death but may lead to diminished efficiency or to pathological conditions. Small variations in the acid base value of the diet probably have little significance provided the total salt content is adequate; for the body can excrete that portion which is not essential. A person or group of persons may take too acid a diet for one period and correct its effects by an alkaline diet at another period: for example, diets acid from meat and cereals in the winter, and diets alkaline from fruit and vegetables in the summer. Groups which select special limited diets, such as nuts, fruit, vegetables, or meat alone would make interesting studies. Acid diets have proven beneficial in infantile tetany and their use has been suggested in rickets. Alkaline diets are essential for infancy where growth is rapid. Their use is strongly indicated in pregnancy and lactation and such pathological conditions as diabetes. From Chittenden's study of athletes and soldiers on vegetable food it appears that on alkaline diets the body is capable of more sustained effort than on acid diets.

Acid-base value of the urine. The urine is the most important means of maintaining the acid-base equilibrium of the body. Through the excretion of acid or alkali the organism maintains a constant or nearly constant condition, whether the food is acid or alkaline. This function is so delicate that the excretion varies from day to night or from hour to hour. During secretion of HCl for digestion, the urine is alkaline. Even the effect of forced breathing, which removes the CO_2 from the blood and makes it more alkaline, is immediately reflected in the secretion of an alkaline urine.

Gaethgens and Stadelman (210) were the first to calculate the acid-base value of the urine. They analyzed the minerals and expressed their values as equivalents of sodium. How this excretion is accomplished and how the factors vary with acidity or alkalinity has recently been studied (81), (167), (225). The urine is normally acid. This acidity depends upon, *1*, the excretion of acid phosphate; *2*, acid which is neutralized by ammonia formation; *3*, organic acids; *4*, acid neutralized by inorganic minerals; *5*, carbonic acid, as bicarbonate and carbonate. Normally the urine contains 500 to 1000 cc. of acid, depending upon the diet. The acid (Palmer) is divided as acid phosphate, 100 to 200 cc., acid neutralized by ammonia, 300 to 400 cc., organic acid, 0 to 150 cc., carbonates, 10 to 40 cc. The free acid represents half the value, and the ammonia, the other half.

The organic acids—uric, hippuric, lactic—are usually small but under varying conditions of diet and exercise they may be 150 to 200. Under pathological conditions they may greatly exceed this value. The phosphoric acid excreted in the urine depends upon the amount in the diet. That portion of the acid excretion which is neutralized by basic minerals is not represented in the values obtained by titration methods, but is only detected when mineral metabolism is investigated. Under normal conditions mineral loss is important. The urine normally excretes nearly all the chloride, 80 per cent of the sodium and potassium and sulphur, 50 per cent of the phosphorus, but only 20 to 30 per cent of the calcium in adults and only 5 to 10 per cent of the calcium in infants. The mineral constitutents of the urine are controlled not only by the diet but also by the osmotic, ionic and acid-base equilibria.

Acid-base value of feces. That the feces play little or no part in normal acid base equilibrium and that the minerals there found represent merely those unabsorbed has been generally assumed. Even if mineral radicals were not excreted or secreted by the bowel, the factors which govern their absorption would control the acid-base equilibrium in the body. The work of Goldschmidt and Dayton (68), (69) indicates that the bowel does play an important part in the transfer of minerals to and from the blood. Measurements of pH of the contents of the small intestines by McClendon and of the feces by Hawk show such constancy of the reaction as to indicate some regulatory process. Further, Ylppö has shown that the pH of the feces after breast milk feeding is acid—about pH 5.0—and after cow's milk feeding is alkaline—about pH 8.0. Other evidence of the regulatory process is shown by a study of the paths of excretion; for example, nearly all the calcium leaves the body through the feces, and the chlorine through the urine.

A method on the titration value of the stools was proposed by Blauberg (178). He studied the amount of acid or alkali necessary to neutralize the dried stool. Similar studies have been made by Talbot and Hill (219). The interpretation of these values is difficult. The relations of acid to base are altered by drying. Even if correct values on twenty-four hour metabolism periods were obtained, they would represents only the unbuffered states—equivalent to the free acid excretion in the urine. They would not represent the total acidity. Other methods are needed.

By analysis of the mineral radicals, expressed in terms of sodium equivalents, Fleitmann (cited by (72)) showed that the stools were alkaline. A normal value for adults is 200 to 300 cc., as determined by v. Wendt, and for the infant, 500 cc. 0.1N base per day. Studies by Shohl and Sato show that the bowel, by excretion of alkali, normally participates in regulation of the acid base equilibrium.

THE EFFECT OF ACID ON METABOLISM. The first important studies on the effects of ingested acid were made at Dorpat. Under the direction of Bucheim appeared a series of papers on the excretion of mineral acids in the urine. In 1877 a classic paper by Walter (230) showed that acid led to poisoning and death. His experiments on dogs demonstrated: *1*, a diminished CO_2 in the blood, and *2*, an increased amount of ammonia in the urine. Salkowski (182) emphasized that especially in herbivora acid caused a loss of alkali minerals in the urine.

Experiments in acid feeding and the resultant condition "acidosis" have developed along three lines:

1. The effect of acid on CO_2 in the blood and other factors in the acid-base equilibrium in the blood. This is reviewed in a forthcoming article by Wilson (237).

2. The effect of acid on the NH_3 in the urine. Controversies have arisen over the difference between carnivora and herbivora and the fate of various nitrogenous elements, but the consensus of opinion of nearly a hundred investigators has substantiated Walter's finding—when acids are given, the urinary NH_3 is increased.

3. The loss of alkali minerals also in the urine of acid feeding has now been well established. When 250 cc. of acid are given, all the acid radicals and also 100 cc. extra bases are excreted in the urine. Stehle and MacCarthy (212) have recently reviewed this question in both dogs and men, as have Givens and Mendel (66), Gamble (62), and Shohl and Sato for infants.

Acid poisoning is produced not only by the mineral acids, hydrochloric, sulphuric and phosphoric, but also by organic acid. Ingested benzoic, lactic or organic acids produced in metabolism have been shown to cause acidosis. Of the latter, β oxybutyric acid in diabetes is quantitatively the most important; as much as 80 grams may be excreted daily. Organic acids are also formed in methyl alcohol poisoning, pneumonia, and in intestinal disorders in infancy.

The mechanism of the action of acids on the urine, calculated on the daily output, shows that, *1*, the acidity of the urine increases up to pH 4.6; *2*, the amount of free acid excreted—measured by the titra-

table acidity—increases up to 2500 cc. 0.1N; *3*, the phosphate is excreted in increased amount, two or threefold, and more is converted to acid phosphate, so the excretion of acid increases up to 700 cc.; *4*, the organic acids may amount to 10 liters 0.1N or more. In the acid urine 30 per cent is excreted as free acids, thus does not require base and ammonia for neutralization. *5*, The ammonia shows the greatest increase. In diabetes it may be as large as 7000 cc. 0.1N; *6*, no accurate data are available for the loss of alkali minerals in extreme acidosis, but, by estimate, it may amount to 500 cc. 0.1N.

That the stools are active in regulation of the acid base equilibrium is becoming more apparent. Shohl and Sato have stated that the stool participates in the regulation of acid base equilibrium and in emergency it may excrete acid. Steinitz and Sawyer, Bauman and Stevens have also shown that the feces may even cause acidosis by excreting alkali.

From the point of view of mineral metabolism, acid causes a diminution in the retention of all the elements, but the effect is greater upon the alkali radicals than the acid radicals. In a baby on a milk diet, when 250 cc. 0.1N HCl (Shohl and Sato) and 750 cc. 0.1N (Gamble) were given, one-seventh of the acid was retained and the rest excreted by the urine. The infant on a diet made neutral by the addition of HCl excretes an excess of acid and retains base. The body further responds to the acids by furnishing alkali from the blood tissues and bones. Acid lowers the alkali reserve through excretion of CO_2 by the lungs. There is a rearrangement of the equilibria in the blood, causing a diminished pH. Acid ingestion results in a diminished base balance or in a negative base balance.

Effect of alkalis on metabolism. Alkalosis is the term used to indicate an increase in the pH of the blood produced by alkalis. Forced breathing, high altitudes and cold baths also have been shown to produce this condition. The effect of alkalis on metabolism of minerals is little known. The only complete metabolism studies which have been published are those of v. Wendt (for a single day) and of Shohl and Sato (for a single period). The latter have emphasized that the bowel can excrete alkalis. Together with the urine, the feces protect the body from alkalosis. The urine becomes alkaline by excreting sodium; the stool by excreting calcium. However, an increased retention of alkalis takes place. This retention affects the water metabolism; a gain in weight or even edema occurs after alkali treatment.

The material on the metabolism of alkalis is not large, though their use in medicine has been extensive since the time of the ancients. A review of the early literature is given by Quenoille (172). Wöhler's researches (239) constitute the beginning of scientific knowledge. He showed that the organic salts of alkalis are changed to carbonates in the body and make the urine alkaline. The early work following Bence Jones' is largely confined to the effect of alkali on the acidity of the urine, or on the nitrogen metabolism. The discovery of β oxybutyric acid by Minkowski (151) in the acid intoxication of diabetes and the recognition by Stadelmann of the increased ammonia in the urine, gave new significance to the effect of alkalis. Stadelmann felt that if an acid intoxication existed, alkalis could be used to neutralize the acids. He therefore carried out, through his students at Dorpat, a series of researches on the effect of alkalis and published, in 1890, (210) a monograph of the work of Burchard, Klemptner, Beckmann, Hagentoren and Kozerski. This work is important and difficult of access. It shows great care in the planning of experiments, in analytical methods, and in the review of the literature.

Burchard determined the urea, uric acid, ammonia and chlorides in the urine, and in some of his experiments the nitrogen in the feces also. On a constant diet he took sodium bicarbonate, increasing from 5.8 grams per day to 27.0 grams per day. The last dose caused a diarrhea and he took small amounts of opium. His conclusions are that alkalis have no dyspeptic action but make the urine definitely alkaline and increase the volume. Taken for a long time, they slightly decrease the nitrogen output; definitely decrease the ammonia output to minimum amounts, they diminish the uric acid. Klemptner repeated and confirmed Burchard's work.

Beckmann took for his part of the study the analysis of sodium, potassium, calcium, magnesium and ammonia. Unfortunately he did not analyze the minerals in the feces. The output of sodium was larger than the intake; in one case the excretion was 150 per cent of the intake. A small loss of potassium and chloride also occurred. The output of calcium and magnesium was not influenced. The ammonia was depressed in proportion to the amount of alkali given. The chlorides excreted are insufficient to account for the excess of sodium, so that it must be excreted in part as carbonate.

Hagentoren also analyzed Beckmann's urine and determined phosphorus, nitrogen, uric acid, total sulphuric acid, neutral sulphur, preformed sulphate, hydrochloric acid. Calculating both acids and

bases including ammonia, as equivalents of sodium, he showed that normal urine contains an excess of base equivalent to 0.49 sodium, but of this the ammonia amounts to 0.58. When alkali is taken, the excess of base is much larger. When sodium citrate is taken, sodium chloride and potassium chloride are withdrawn from the body in proportion to the amount of salt taken. With sodium bicarbonate, chlorine is not removed. The output of sulphur or phosphorus is not affected. Neutral sulphur is increased. Sodium bicarbonate and sodium citrate are in part excreted as carbonates.

Kozerski analyzed sodium and potassium by improved methods, and attempted to discover whether sodium citrate differs from carbonate in its effect upon the urine. He, too, was on a weighed and measured diet, and determined not only the urine but also the nitrogen of the feces. He unfortunately did not determine any of the bases in the feces. Amounts of sodium larger than 7.0 grams increase the output of potassium and chloride. The organism eventually diminishes the excessive output of sodium and chloride. When the alkali is stopped, the urine becomes acid on the second day and the chloride and potassium output become normal. Alkali causes no loss of nitrogen. Sodium carbonate has exactly the same action which Beckmann found for citrate. The output of sodium increases as the intake, so that large doses bring about a loss of sodium from the body.

The effect of alkalis on uric acid has been much discussed in the literature. It led to the practice so much in vogue fifty years ago of giving alkalis, especially lithium salts, to drive excess of uric acid from the body. Lithium urate is the most soluble salt of uric acid but it does not prevent the insoluble forms of the salt from precipitating in joints of patients with gout. Alkaline urine does dissolve uric acid. The amount of uric acid held in solution is a function of the pH.

Phosphates in the urine created a similar problem. Acid urines hold phosphates in solution; alkaline urines do not. The effect upon the elimination of phosphates by the body depends upon the balance. Alkali causes less phosphate to be excreted by the urine and more by the bowel. In infants the total phosphorus excretion is increased by both acids and alkalis. The action of alkalis can be summarized. In the urine *1*, the reaction becomes alkaline up to pH. 8.4—which results in a negative value for the titratable acid up to 200 cc. *2*, The ammonia is diminished to minimal amounts and may actually be eliminated. This increases alkali excretion up to 500 cc. 0.1N. *3*, The phosphate is converted to alkaline phosphate, an increase of alkali

excretion up to 400 cc. 0.1N. *4*, The organic acids, if present, are completely neutralized. *5*, The carbonates may be present up to 1000 cc. for neutralization of base. *6*, The chlorine and potassium are slightly increased. The alkali excretion is accomplished by means the opposite of those in acid excretion. In acid excretion the pH is diminished and the acidity regulated by the phosphates. Ammonia is increased to neutralize the acid. Carbonate is negligible. In alkali excretion the pH is increased and the acidity regulated by the carbonates. Ammonia is negligible. Carbonate is formed to neutralize the base.

In the feces there is an increased excretion of alkali, mainly as soaps, phosphates and carbonates of calcium. This together with the urine removes excess alkali.

In the body alkali retention is increased, which causes an increase in body fluids and also makes the blood more alkaline.

Conclusions. What is the significance of the requirement of minerals in health? They furnish no energy. They are not consumed. Why does the body waste them? Is there any significance in the amount stated as a requirement? If the diet were altered the requirement might also be altered. Rubner (cited by Greenwald) states that the Japanese diet is adequate, although low in calcium, because it is also low in fat. If it were low in phosphate, could it not also be low in calcium? In other words, a relationship exists between each mineral element in the diet to the total caloric intake, to the organic factors, to each of the other mineral constituents and to the acid-base balance of the body.

In the physiologic literature of the last twenty-five years the relationship of one element to another has been made clear, especially its significance for the mechanism of the heart beat, of nerve stimulation, etc. Quite recently Loeb has shown the new significance of the ionic relations in physiology, according to the Donnan equilibrium.

Not only as regards amounts are minerals of great significance in relation to one another, but also as regards concentration. Their concentration determines the osmotic pressure relationships of the body and hence the water metabolism. In order to solve the problem in any real sense, to establish the requirements of any one element, all others must be held constant while that one is varied which gives a number of permutations.

In growth and pregnancy and in cases of neoplasms, minerals must be retained. In adult life an equilibrium should exist. Even under

the most favorable conditions a loss from the body and replacement by food continually occurs. No satisfactory explanation for the mineral flux has ever been offered. A possible approach to the problem may be an understanding of the significance of the "alkaline tide" observed by Bence Jones.

The minerals, therefore, aid in maintaining at least three kinds of equilibria: *1*, osmotic, *2*, ionic, and *3*, acid-base. A normal standard of base balance in the human body has been defined. The effect upon the base balance has been determined for alkalosis and acidosis.

SUMMARY

1. Mineral metabolism can be studied simply and effectively in terms of normal solutions. Measurement of the acid-base balance is thus made possible.

2. The normal mineral requirements per man per day approximate 150 cc. 0.1N acid.

3. The fetus shows a retention of alkali for the last one hundred days of gestation equal to 67.0 cc. 0.1N alkali per day.

4. The infant and child show a positive base balance amounting to 10 cc. 0.1N base per kilo per day.

5. The adult balance approximates equilibrium.

6. Pregnancy and lactation require additional alkali—a minimum of 150 cc. 0.1N base per day.

7. Acid diminishes a positive balance or causes a negative base balance.

8. Alkali increases the positive base balance.

BIBLIOGRAPHY

(1) Adler, H. M. and G. Blake. The retention of alkali by the kidney with special reference to acidosis. Arch. Int. Med., 1911, vii, 479.

(2) Adler, Z. Über den Einfluss der Alkalien auf den Kalkumsatz beim Kinde. Monatschr. f. Kinderh., 1906, v, 180.

(3) Albu, A. and C. Neuberg. Physiologie und Pathologie des Mineralstoffwechsels. Berlin, 1906; *also* p. 28 references 1–17.

(4) Aron, H. Die Verluste des Säuglings im Hunger. J. f. Kinderheilk., lxxxvi, 128.

(5) Aron, H. and M. Franz. Organische Säuren im Säuglingsharn. Monatschr. f. Kinderh., Orig., 1914, xii, 645.

(6) Aschenheim, E. Beitrag zum Fett-, Kalk- und Stickstoffwechsel beim Säugling. Jahrb. f. Kinderh., 1913, lxxvii, 505.

(7) Bahrdt, H. et al. Untersuchungen über die Pathogenese der Verdauungsstörungen im Säuglingsalter. I–VIII. Zeitschr. f. Kinderh., 1912, iv, 534; 1912–13, v, 475; 1914, xi, 143.

(8) Benedict, F. G. A study of prolonged fasting. Washington, Carnegie Inst., 1915. Carnegie Inst., Pub. no. 203.

(9) von Bibra, E. Chemische Untersuchungen über die Knochen und Zähne des Menschen und der Wirbelthiere, mit Rücksichtnahme auf ihre physiologischen und pathologischen Verhältnisse. Schweinfurt, Kunstverlag, 1844.

(10) Berg, R. Die Nahrungs- und Genussmittel; ihre Zusammensetzung, mit besonderer Berücksichtigung der Aschenbestandteile. Dresden, 1913.

(11) Bernard, C. Des Differences que présentent les phénomènes de la digestion et de la nutrition chez les animaux herbivores et carnivores. Comp. rend. Acad. d. sci., 1846, xxii, 534.

(12) Birk, W. Beiträge zur Physiologie des neugeborenen Kindes. Die Bedeutung des Kolostrums. Analysen und Stoffwechselversuche. Monatschr. f. Kinderh., Orig., 1910, ix, 595.

(13) Birk, W. Über den Magnesiumumsatz des Säuglings. Jahrb. f. Kinderh., 1907, lxvi, 300.

(14) Birkner, K. and R. Berg. Untersuchungen über den Mineralstoffwechsel I. Entfettungskuren. Zeitschr. f. klin. Med., 1913, lxxvii, 471.

(15) Blane, G. On the effect of the pure fixt alkalies and of lime water, in several complaints. Trans. Soc. Improve. M. and Chir. Knowl., 1800, ii, 132.

(16) Blatherwick, N. R. The specific rôle of foods in relation to the composition of the urine. Arch. Int. Med., 1914, xiv, 409.

(17) Blatherwick, N. R. Note on the acid-base of army rations. Amer. Journ. Physiol., 1919, xlix, 567.

(18) Blatherwick, N. R. Neutrality regulation in cattle. Journ. Biol., 1920, xlii, 517.

(19) Blauberg, M. Ueber die Mineralbestandtheile der Säuglingsfäces bei natürlicher und künstlicher Ernährung während der ersten Lebenswoche. München, 1897. Diss.

(20) Blauberg, M. Experimentelle Beiträge zur Frage über den Mineralstoffwechsel beim künstlichernährten Säugling. Zeitschr. f. Biol., 1900, N.F. xxii, 1.

(21) Blauberg, M. Ueber den Mineralstoffwechsel beim natürlichernährten Säugling. Ztschr. f. Biol., 1900, N.F. xxii, 36.

(22) Brubacher, H. Ueber den Gehalt an anorganischen Stoffen, besonders an Kalk, in den Knochen und Organen normaler und rhachitischer Kinder. Zeitschr. f. Biol., 1890, N.F. ix, 517.

(23) Bruck, A. W. Über den Mineralstoffwechsel beim künstlichgenährten Säugling. Monatschr. f. Kinderh., 1907, vi, 570.

(24) Buchheim, R. Lehrbuch der Arzneimittellehre. 3. Aufl. Leipzig, 1878.

(25) Bunge, G. Der Kali-, Natron- und Chlorgehalt der Milch, verglichen mit dem anderer Nährungsmittel und des Gesammtorganismus der Säugethiere. Dorpat, 1874. Diss.

(26) Bunge, G. Über die Bedeutung des NaCl and des KCl im menschlichen Organismus. Zeitschr. f. Biol., 1873, ix, 104.
(27) Camerer, W., Jr. Die chemische Zusammensetzung des neugeborenen Menschen. Zeitschr. f. Biol., 1900, xxxix, 178; 1902, xliii, 1.
(28) Caulet. De la suralcalisation du sang et des urines sous l'influence de la chaux et de la magnésie. Bull. gén. de thérap., 1875, lxxxviii, 349; 399.
(29) Clare, W. Experimenta de excretione acidi sulfurici per urinam. Dorpat, 1854. Diss.
(30) Coranda. Über das Verhalten des Ammoniaks im menschlichen Organismus. Arch. f. exper. Path. u. Pharm., 1879, xii, 76.
(31) Chittenden, R. H. The nutrition of man. N.Y., 1907, 321 p.
(32) Czerny, A. and A. Keller. Des Kindes Ernährung, Ernährungsstörungen und Ernährungstherapie. Wien, 1906, i, Bd.
(33) Delarand. Recherches sur les variations de l'acidité de l'urine aux différentes émissions du jour. Comp. rend. Soc. de biol., 1851, 1852, iii, 118.
(34) Denis, W. and A. S. Minot. Ammonia excretions as influenced by the ingestion of alkalies. Journ. Biol. Chem., 1918, xxxv, 101.
(35) Dennstadt, M. and T. Rumpf. Über die Bestimmung der anorganischen Bestandteile in menschlichen Organen. Hoppe-Seyler's Zeitschr. f. physiol. Chem., 1904, xli, 42.
(36) Dorlencourt, H. and M. Delort. Rôle de la substance minérale dans la nutrition normale et pathologique du nourrisson. Nourisson, 1914, ii, 217.
(37) Drouin, R. Hémo-alcalimétrie, hémo-alcidimétrie; étude des variations de la réaction alcaline et de l'acidite reélle du sang dans les conditions physiologiques et pathologiques. Paris, 1892. Thesis.
(38) Dubois, M. and K. Stolte. Abhängigkeit der Kalkbilanz von der Alkalizufuhr. Jahrb. f. Kinderh., 1913, lxxvii, 21.
(39) Dunlop, J. C. On the action of large doses of dilute mineral acid on metabolism. Journ. Physiol., 1896, xx, 82.
(40) Emmerisch, R. and O. Loew. Über Kalkmangel in der menschlichen Nahrung. Zeitschr. f. Hyg. u. Infectionskrankh., 1914, lxxii, 311.
(41) Eylandt, T. De acidorum sumptorum vi in urinae acorem. Dorpat, 1854.
(42) Fauvel, P. Action de l'acide chlorhydrique sur l'excrétion urique. Compt. rend. Soc. de biol., 1908, lxiv, 736.
(43) Fauvel, P. Action du bicarbonate de soude sur l'excrétion urique (régime sans purines). Compt. rend. Soc. de biol., Paris, 1908, lxiv, 557.
(44) Fehling, H. Beiträge zum Physiologie des placentaren Stoffverkehrs. Arch. f. Gynaek., 1877, xi, 523.
(45) Fiske, C. H. Inorganic phosphate and acid excretion in the postabsorptive period. Journ. Biol. Chem., 1921, xlix, 171.
(46) Fiske, C. H. Observations on the "alkaline tide after meals." I. Journ. Biol. Chem., 1921, xlix, 163.
(47) Fiske, C. H. Relation between ammonia excretion and the hydrogen ion concentration of urine. Proc. Amer. Soc. Biol. Chemists (Journ. Biol. Chem., 1920, xli, xxxix).

(48) Forbes, E. B., J. A. Schulz, C. H. Hunt, A. R. Winter and R. F. Remler. The mineral metabolism of the milch cow. Journ. Biol. Chem., 1922, lii, 281.

(49) Forbes, E. B. and H. Keith. A review of the literature of phosphorus compounds in animal metabolism. Ohio Agric. Exper. Sta., 1919, Tech. Ser. Bull., v, 1.

(50) Forbes, E. B., J. O. Halverson, L. E. Morgan and J. A. Schulz. The metabolism of calcium compounds by growing swine. I–II. Ohio Agric. Exper. Sta. Bull., 1921, cccxlvii, 3.

(51) Forbes, E. B. The balance between inorganic acids and bases in animal nutrition. Ohio Agric. Exper. Sta. Bull., 1909, ccvii, 23.

(52) Forster, J. Versuche über die Bedeutung der Aschebestandtheile in der Nahrung. Zeitschr. f. Biol., 1873, ix, 297.

(53) Forster, J. Ueber die Verarmung des Körpers, speciell der Knochen an Kalk bei ungenügender Kalkzufuhr. Zeitschr. f. Biol., 1876, xii, 464.

(54) Fuhage, G. Untersuchungen über den Einfluss des Basensäurenverhältnisses auf den Eiweissbedarf. Arch. f. Kinderh., 1919, lxvii, 291.

(55) Freund, W. Physiologie und Pathologie des Fettstoffwechsels im Kindesalter. Ergbn. d. inn. Med. u. Kinderh., 1909, iii, 139.

(56) Freund, W. Zur Wirkung der Fettdarreichung auf den Säuglingsstoffwechsel. Jahrb. f. Kinderh., 1905, lxi, 36.

(57) von Fürth, O. The problems of physiological and pathological chemistry of metabolism. Transl. by Allen J. Smith. Philadelphia and London, 1916.

(59) Gaehtgens, C. Über Ammoniak-Ausscheidung. Zeitschr. f. physiol. Chem., 1880, iv, 36.

(60) Gamble, J. L. Review of recent literature on conditions of abnormal metabolism in infants. Amer. Journ. Dis. Child., 1917, xiii, 362.

(61) Gamble, J. L. Carbonic acid and bicarbonate in urine. Journ. Biol. Chem., 1922, li, 295.

(62) Gamble, J. L. Studies in tetany. I and II. (To appear.)

(63) Gamble, J. L. Mineral metabolism in starvation. Read before the Society for Clinical Investigation, Washington Meeting, 1921. (To appear.)

(64) Giffhorn, H. Beiträge zur Kenntnis des Stoffwechsels, besonders der Mineralien, im Säuglingsalter. III. Der Einfluss von Fettzulagen auf den Stoffwechsel-verdauungs gesunder Kinder bei molkenarmer und molkenreicher Ernährung. Jahrb. f. Kinderh., 1913, lxxviii, 531.

(65) Givens, M. H. Studies in calcium and magnesium metabolism. III. The effect of fat and fatty acid derivatives. Journ. Biol. Chem., 1917, xxxi, 441.

(66) Givens, M. H. and L. B. Mendel. Studies in calcium and magnesium metabolism. I. The effects of base and acid. Journ. Biol. Chem., 1917, xxxi, 421.

(67) Görger, T. Ueber die unter physiologischen Bedingungen eintretende Alkalescenz des Harns. Arch. f. exper. Path. u. Pharm., 1879, xi, 156.

(68) Goldschmidt, S. and C. Binger. Studies in the mechanism of absorption from the intestine. VI. Amer. Journ. Physiol., 1919–20, xlviii, 473.

(69) GOLDSCHMIDT, G. AND A. B. DAYTON. Studies in the mechanism of absorption from the intestine. I–V. Amer. Journ. Physiol., 1919–20, xlviii, 419.

(70) GOODALL, H. W. AND E. P. JOSLIN. Experiments with an ash-free diet. Trans. Assoc. Amer. Physicians, 1908, xxiii, 92.

(71) GOTO, K. Mineral metabolism in experimental acidosis. Journ. Biol. Chem., 1918, xxxvi, 355.

(72) GRUNDZACH, J. Über die Asche des normalen Kothes. Zeitschr. f. klin. Med., 1893, xxiii, 70.

(73) GREENWALD, I. A normal diet. In: Endocrinology and metabolism, ed. by L. F. BARKER. New York and London, 1922, iii.

(74) GUTTMANN, P. Über die Wirkung einiger Sämen bei uhrer Injection in die Venen. Virchow's Arch. f. path. Anat., 1877, lxix, 534.

(75) HALLERVORDEN, E. Über das Verhalten des Ammoniaks in Organismus und seine Beziehung zur Harnstoffbildung. Arch. f. exper. Path. u. Pharm., 1878, x, 125.

(76) HASSELBACH, K. A. Ein Beitrag zur Respirations physiologie der Gravidität. Skand. Arch. f. Physiol., 1912, xxvii, 1.

(77) HASSELBACH, K. A. AND S. A. GAMMELTHOF. Die Neutralitätsregulation des graviden Organismus. Biochem. Zeitschr., 1915, lxxviii, 206.

(78) HEITZMANN, C. Ueber künstliche Hervorrufung von Rhachitis und Osteomalacie. Allg. Wien. med. Ztg., 1873, xviii, 570.

(79) HENDERSON, L. J. Acidosis and the physico-chemical equilibrium of the organism. In: Oxford medicine, London and New York, 1919, i, 471.

(80) HENDERSON, L. J. Das Gleichgewicht zwischen Basen- und Säuren im tierischen Organismus. Ergbn. Physiol., 1909, viii, 254.

(81) HENDERSON, L. J. A critical study of the process of acid excretion. Journ. Biol. Chem., 1911, ix, 403.

(82) HENDERSON, L. J. AND W. W. PALMER. On the extremes of variation of the concentration of ionized hydrogen in human urine. Journ. Biol. Chem., 1913, xiv, 81.

(83) HENDERSON, L. J. AND W. W. PALMER. On the intensity of urinary acidity in normal and pathological conditions. Journ. Biol. Chem., 1913, xiii, 393.

(84) HERBST, O. Beiträge zur Physiologie des Stoffwechsels im Knabenalter mit besonderer Berücksichtigung einiger Mineralstoffe. Jahrb. f. Kinderh., 1912, lxxvi, 40.

(85) HINDHEDE, M. Harnsäurelösende Diät. Zeitschr. f. phys. u. diätet. Therap., 1913, xvii, 592.

(87) HIS, W., JR. Die Ausscheidung von Harnsäure im Urin der Gichtkranken, mit besonderer Berücksichtigung der Anfallszeiten und bestimmter Behandlungsmethoden. Deutsch. Arch. f. klin. Med., 1900, lxv, 156.

(88) HIS, W., JR. AND T. PAUL. Physikalisch-chemische Untersuchungen über das Verhalten der Harnsäure und ihrer Salze in Lösungen. 2. Abhandlung: Die vermeintliche Leichtlöslichkeit der Harnsäure in wässerigen Lösungen starker Säuren. Hoppe-Seyler's Zeitschr. f. physiol. Chemie, 1900, xxxi, 64.

(89) Hofmann, F. Über den Übergang von freien Säuren durch das alkalische Blut in den Harn. Zeitschr. f. Biol., 1871, vii, 338.

(90) Holt, L. E., A. M. Courtney and H. L. Fales. The chemical composition of diarrheal as compared with normal stools in infants. Amer. Journ. Dis. Child., 1915, ix, 213.

(91) Hornemann. Zur Kenntnis des Salzgehaltes der täglichen Nahrung des Menschen. Zeitschr. f. Hyg. u. Infectionskrankh., 1913, lxxv, 553.

(92) Hougardy, A. Étude de l'action physiologique de quelques substances à réaction alcaline. Arch. internat. de Pharmacod., 1904, xiii, 91.

(93) Howe, P. E. and P. B. Hawk. Studies on water drinking: XIII. (Fasting studies: VIII.) Hydrogen ion concentration of feces. Journ. Biol. Chem., 1912, xi, 129.

(94) Howland, J. and W. M. Marriott. A discussion of acidosis, with special reference to that occurring in diseases of children. Johns Hopkins Hosp. Bull., 1916, xxviii, 63.

(95) Howland, J. and W. M. Marriott. Acidosis occurring with diarrhea. Amer. Journ. Dis. Child., 1916, xi, 309.

(96) Hübschman, F. De alcali primigenio. Erlangae, 1761. Diss.

(97) Hugounenq, L. Recherches sur la composition minérale de l'organisme chez le foetus humain et l'enfant nouveau. Journ. Physiol. et Pathol. gén, 1899, i, 703.

(98) Hugounenq, L. La statique minérale du foetus humain pendant les cinq derniers mois de la grossesse. (3e mémoire). Journ. Physiol. et Pathol. gén., 1900, ii, 509.

(99) Hugounenq, L. Statique minérale du foetus humain, pendant les cinq derniers mois de la grossesse. Compt. rend. Acad. d. Sci., 1900, cxxx, 1422.

(100) Hugounenq, L. Sur la fixation des bases alcalines dans le squelette minéral du foetus pendant les cinq derniers mois de la grossesse. Compt. rend. Acad. d. Sci., 1900, cxxx, 941.

(101) Hutchison, H. S. Fat metabolism in health and disease with special reference to infancy and childhood. Quart. Journ. Med., 1919–20, xiii, 277.

(102) Janney, N. Die Ammoniakausscheidung im menschlichen Harne bei Zufuhr von Harnstoff und Natron. Hoppe-Seylers Zeitschr. f. physiol. Chem., 1911, xii, 99.

(103) Jansen, W. H. Zur Frage der Abhängigkeit des Eiweisstoffwechsels vom Säuren- Basengehalt der Nahrung. Zeitschr. f. klin. Med., 1919, lxxxviii, 221.

(104) Jawein, G. Zur Frage über den Einfluss des doppeltkohlensauren resp. citronensauren Natriums, in grossen Dosen gegeben, auf den Stickstoffumsatz, sowie auf die Menge des neutralen Schwefels und der Astherschwefelsäuren des Harns beim gesunden Menschen. Zeitschr. f. klin. Med., 1895, xxii, 43.

(105) Jones, H. B. On animal chemistry in its application to stomach and renal diseases. London, 1850.

(106) Katz, J. Die mineralischen Bestandtheils des Muskelfleisches. Arch. f. d. gesammt. Physiol., 1896, lxiii, 1.

(107) KEETON, R. W. Ammonia excretion following experimental administration of acids via the stomach and peripheral vein. Journ. Biol. Chem., 1921, xlix, 411.

(108) KELLER, A. Zur Kenntnis der Gastroenteritis im Säuglingsalter. II. Ammoniakausscheidung. Jahrb. f. Kinderh., 1897, xliv, 25.

(109) KELLER, A. Zur Kenntnis der chronischen Ernährungsstörung der Säuglinge. III. Fettumsatz und Azidose. Monatschr. f. Kinderh., 1902, i, 234.

(110) KELLER, A. Über den Einfluss der Zufuhr anorganischer Säuren auf den Stoffwechsel des Säuglings. Centralbl. f. allg. Path. u. path. Anat., 1898, viii, 947.

(111) KLEIN, W. AND F. MORITZ. Das Harnammoniak beim gesunden Menschen unter dem Gesichtspunkt einer ausschliesslich neutralisatorischen Funktion desselben, sowie die Bilanzverhältnisse Zwischen Säuren und Alkalien im menschlichen Harn bei verschiedener Ernährung. Deutsch. Arch. f. klin. Med., 1910, xcix, 162.

(112) KLOTZ, M. Milchsäure und Säuglingsstoffwechsel. Jahrb. f. Kinderh., 1909, lxx, 1.

(113) KLOSE, E. Zur Kenntnis der Körperzusammensetzung bei Ernährungsstörungen. Jahrb. f. Kinderh., 1914, lxxx, 154.

(114) KOEPPE, H. Studien zum Mineralstoffwechsel. Jahrb. f. Kinderh., 1913, lxxiii, 9.

(115) LABBÉ, H. AND L. VIOLLE. Ingestion d'acides minéraux chez le chien. Compt. rend. Acad. d. Sci., 1911, clii, 279.

(116) LAMB, A. R. AND J. M. EVVARD. Acid-base balance in animal nutrition. II. Metabolism studies on the effect of certain organic and mineral acids on swine. Journ. Biol. Chem., 1919, xxxvii, 329.

(117) LAMB, A. R. AND J. M. EVVARD. Acid-base balance in animal nutrition. I. The effect of certain organic and mineral acids on the growth, well-being and reproduction of swine. Journ. Biol. Chem., 1919, xxxvii, 317.

(118) LANDSBERG, E. Eiweiss und Mineralstoffwechsel Untersuchungen bei der schwangeren Frau nebst Tierversuchen mit besonderer Berücksichtigung der Funktion endokriner Drüsen. Zeitschr. f. Geburtsh. u. Gynäk., 1914, lxxvi, 53.

(119) LANDSBERG, E. Untersuchungen über den Stoffwechsel von Stickstoff, Phosphor und Schwefel bei Schwangeren. Zeitschr. f. Geburtsh. u. Gynäk., 1912, lxxi, 163.

(120) LANGE, C. DE. Die Zusammensetzung der Asche des Neugeborenen und der Muttermilch. Zeitschr. f. Biol., 1900, N.F. xxii, 526.

(121) LANGSTEIN, L. AND F. EDELSTEIN. Die chemische Zusammensetzung frühgeborenen Säuglinge und ihre Wachstumansatz. Zeitschr. f. Kinderh., 1916, xv, 49.

(122) LANGSTEIN, L. Untersuchungen über die Acidität und den Zuckergehalt von Säuglingastühlen. Jahrb. f. Kinderh., 1902, lvi, 350.

(123) LANGSTEIN, L. AND L. F. MEYER. Säuglingsnährung und Säuglingsstoffwechsel. 2nd and 3rd ed., 1914. Wiesbaden.

(124) LAVERAN, C. L. A. AND J. TEISSIER. Nouveaux éléments de pathologie médicale. Paris, 1889, i, 773.

(125) Liebig, J. Animal chemistry or organic chemistry in its applications to physiology and pathology. Philadelphia, 1843.
(126) Liebig, J. Chemistry in its application to agriculture and physiology. Philadelphia, 1843.
(127) von Liebig, J. Chemische Untersuchung über das Fleisch und seine Zubereitung zum Nahrungsmittel. Heidelberg, 1847.
(128) Löffler. Cited by Quenouille, Schmidt's Jahrbüch., 1864, 15.
(129) Loewi, O. Arzneimittel und Gifte in ihrem Einfluss auf den Stoffwechsel. In: Handbuch der Pathologie des Stoffwechsels, hrsg. von C. von Noorden. 2. Aufl. Berlin, 1906–07, ii.
(130) Loewi, O. Drugs and poisons. In: Metabolism and practical medicine, by K. H. von Noorden. Eng. ed. by I. W. Hall. London, 1907, iii.
(131) Lohrer, J. Ueber den Uebergang der Ammoniaksalze in den Harn. Dorpat, 1862. Diss.
(132) Lunin, N. Ueber die Bedeutung der anorganischen Salze für die Ernährung des Thieres. Hoppe-Seylers Zeitschr. f. physiol. Chemie, 1881, v, 31.
(133) Magnus-Alsleben, E. Zur Kenntnis der Säuren im Harn. Zeitschr. f. klin. Med., 1911, lxxiii, 428.
(134) Magnus-Alsleben, E. Ueber die Ausscheidung des Kohlenstoffs im Harn. Zeitschr. f. klin. Med., 1909, lxviii, 358.
(135) Magnus-Levy, A. Über den Gehalt normaler menschlicher Organe an Chlor, Calcium Magnesium und Eisen, sowie an Wasser, Eiweiss und Fett. Biochem. Zeitschr., 1910, xxiv, 363.
(136) Maley, R. Untersuchungen über die Quelle der Magensaftsäure. Ann. d. Chem., 1874, clxxiii, 227.
(137) Marshall, E. K. The effect of loss of carbon dioxide on the hydrogen ion concentration of urine. Journ. Biol. Chem., 1922, li, 3.
(138) Martin-Damourette and Hyades. Des effets nutritifs du bicarbonate de potasse à doses modérées. Journ. de thérap., 1880, vii, 561. Also: transl. Bol. de med. nar., San Fernando, 1880, iii, 179.
(139) Martin-Damourette and Hyades. Note sur quelques effets nutritifs des alcalines, à doses modérées, d'après l'experimentation sur l'homme dans l'état de santé. Journ. de thérap., 1880, vii, 441.
(140) Mattill, H. A. and H. I. Mattill. Mineral metabolism. In: Endocrinology and metabolism, ed. by L. F. Barker. New York and London, 1922, iii.
(141) Mauricet, G. Recherches expérimentales pour servir à l'histoire thérapeutique des alcalines. Paris, 1862. Thesis.
(142) Meyer, C. Zur Kenntnis des Mineralstoffwechsels bei der Rachitis. Jahrb. f. Kinderh., 1913, lxxvii, 28.
(143) Meyer, L. F. Die Acidose des Säuglings. Jahrb. f. Kinderh., 1906, lxiii, 30.
(144) Meyer, L. F. Die Bedeutung der Mineralsalz bei den Ernährungsstörungen des Säuglings. Jahrb. f. Kinderh., 1910, lxxi, 1.
(145) Meyer, L. F. Ernährungsstörungen und Salzstoffwechsels beim Säugling. Ergbn. d. inn. Med. u. Kinderh., 1908, i, 317.

(146) Meyer, L. F. Über den Stoffwechsels bei den alimentären Dekomposition. Jahrb. f. Kinderh., 1910, lxxi, 379.

(147) Meyerhofer. Verhandl. der deutsch. Naturforsch. & Ärzte, Karlsbad, 1. Cit. by Albu and Neuberg, 1906.

(148) Mialhe. Nouvelles recherches sur le rôle des alcalines dans l'économie animale. Bull. Acad. d. méd., 1877, 2, ser., vi, 1041.

(149) Michel, C. Sur la composition clinique de l'embryon et du foetus humains aux différentes périodes de la grossesse. Compt. rend. Soc. de Biol., 1899, li, 422.

(150) Miguel, R. Einiges über die Wirkung der Schwefelsäure auf den thierischen Organismus. Arch. f. physiol. Heilk., 1851, x, 479.

(151) Minkowski. Über das Vorkommen von Oxybuttersäure im Harn bei Diabetes mellitus. Ein Beitrag zur Lehre vom Coma diabeticum. Arch. f. exper. Path. u. Pharm., 1884, xviii, 35; 147.

(152) Morawitz, P. Pathologie des Wasser- und Mineralstoffwechsels. In: Handbuch der Biochemie des Menschen und der Tiere, hrsg. von C. Oppenheimer., Jena, 1908–11, iv, Abt. ii.

(153) Müller, E. Über Ernährung debiler Kinder mit molkenreduzierter Milch an der Hand von Stoffwechselsuntersuchungen. Jahrb. f. Kinderh., 1913, lxxiii, 252. (Erg.-Heft.)

(154) Müller, E. and E. Schloss. Beiträge zur Kenntnis des Stoffwechsels besonders der Mineralien im Säuglingsalter. Jahrb. f. Kinderh., 1913, lxxvii, 635.

(155) McClendon, J. F., L. C. Culligan, C. S. Gydesen and F. J. Myers. Relative length of the intestine is more important than the character of the food in determining the hydrogen ion concentration of intestinal contacts. Proc. Amer. Soc. Biol. Chemists (Journ. Biol. Chem., 1920, xli, vi).

(156) McClendon, J. F., F. J. Myers, L. C. Culligan and C. S. Gydesen. Factors influencing the hydrogen ion concentration of the ileum. Journ. Biol. Chem., 1919, xxxviii, 535.

(157) McClendon, J. F., A. Shedlor and B. Karpman. Hydrogen ion concentration of contents of small intestine. Journ. Biol. Chem., 1918, xxxiv, 1.

(158) McClendon, J. F., A. Shedlor and W. Thomson. The hydrogen ion concentration of the ileum content. Journ. Biol. Chem., 1917, xxxi, 269.

(159) McCollum, E. V. and M. Davis. The influence of the composition and amount of the mineral content of the ration on growth. Proc. Amer. Soc. Biol. Chemists (Journ. Biol. Chem., 1913, xiv, xl).

(160) McCollum, E. V. and D. R. Hoagland. Studies of the endogenous metabolism of the pig as modified by various factors. I–III. Journ. Biol. Chem., 1913–14, xvi, 299.

(161) Nelson, C. F. and J. L. Williams. The urinary and fecal output of calcium in normal men together with observations on the hydrogen ion concentration of urine and feces. Journ. Biol. Chem., 1916–17, xxviii, 231.

(162) von Noorden, K. H. and C. Dapper. Mineral waters and metabolism. In: Metabolism and practical medicine, by K. H. von Noorden. Eng. ed. by I. W. Hall. London, 1907, iii, 944.

(163) Osborne, T. B. and L. B. Mendel. Inorganic elements of nutrition. Journ. Biol. Chem., 1918, xxxiv, 131.

(164) Palmer, W. W. Acidosis and acid excretion in pneumonia. Journ. Exper. Med., 1917, xxvi, 495.

(165) Palmer, W. W. and D. D. Van Slyke. Studies of acidosis. IX. Relationship between alkali retention and alkali reserve in normal and pathological individuals. Journ. Biol. Chem., 1917, xxxii, 499.

(166) Palmer, W. W. and L. J. Henderson. On the retention of alkali in nephritis. Journ. Biol. Chem., 1915, xxi, 57.

(167) Palmer, W. W. and L. J. Henderson. Clinical studies on acid base equilibrium and the nature of acidosis. Arch. Int. Med., 1913, xii, 153.

(168) Parkes, E. A. The action liquor potassae on the urine, in health. Brit. & For. M. Chir. Rev., 1853, xi, 201.

(169) Parkes, E. A. The action of liquor potassae on the urine in some chronic diseases. Brit. & For. M. Chir. Rev., 1854, xiv, 383.

(170) Pflaundler, M. Zur Frage der "Säurevergiftung" beim chronisch magendarmkranken Säugling. Jahrb. f. Kinderh., 1904, lx, 719.

(171) Proscher, F. Die Beziehungen der Wachstumsgeschwindigkeit des Säuglings zur Zusammensetzung der Milch bei verschiedenen Säugethieren. Hoppe-Seylers Zeitschr. f. physiol. Chem., 1897, xxiv, 285.

(172) Quenouille, J. A. Considérations générales sur l'action physiologique et thérapeutique des Alcalins. Paris, 1864. Thesis.

(173) Roberts, W. A contribution to urology, embracing observations on the diurnal variations in the acidity of the urine, chiefly in relation to food. Lit. & Phil. Soc., 1859, xv, 238.

(174) Rothberg, O. Über den Einfluss der organischen Komponenten (Eiweiss, Fett, Kohlehydrate) auf den Kalkumsatz künstlich genährter Säuglinge. Jahrb. f. Kinderh., 1907, lxvi, 69.

(175) Rubner, M. and O. Heubner. Die natürliche Ernährung eines Säuglings. Zeitschr. f. Biol., 1898, N.F., xviii, 1.

(176) Rubner, M. and O. Heubner. Die künstliche Ernährung eines normalen und eines atrophischen Säuglings. Zeitschr. f. Biol., 1899, xxxviii, 315.

(177) Rubner, M. and M. Blauberg. Experimentelle und kritische Studien über Säuglinsfeces bei natürlichen und künstlichen Ernährung. Berlin, 1897.

(178) Rüdel, G. Ueber die Resorption und Ausscheidung des Kalkes. Arch. f. exper. Path. u. Pharm., 1894, xxxiii, 79.

(179) Salkowski, E. Über die Wirkung der Säuren im Organismus. Arch. f. exper. Path. u. Pharm., 1877, vii, 421.

(180) Salkowski, E. and I. Munk. Über die Beziehungen der Reaction des Harns zu seinem Gehalt an Ammoniaksalzen. Virchow's Arch. f. path. Anat., 1877, lxxi, 500.

(181) Salkowski, E. Bemerkungen über die Wirkung der unorganischen Säuren und der Fleischnahrung. Virchow's Arch. f. path. Anat., 1879, lxxvi, 368.

(182) Salkowski, E. Über die Möglichkeit der Alkalientziehung beim lebenden Thier. Virchow's Arch. f. path. Anat., 1873, lviii, 1934.

(183) Salkowski, E. Uber die Grösse der Harnsäureausscheidung und der Einfluss der Alkalien auf dieselbe. Virchow's Arch. f. path. Anat., 1889, cxvii, 570.

(184) Sawyer, M., L. Baumann and F. Stevens. Studies of acid production. II. The mineral loss during acidosis. Journ. Biol. Chem., 1918, xxxiii, 103.

(185) Sawyer, M., F. A. Stevens and L. Baumann. Studies of acid production. I. The varying susceptibility of children to acidosis. A preliminary report. Amer. Journ. Dis. Child., 1918, xv, 1.

(186) Scheer, K. Über die Ursachen der Acidität der Säuglingsfaeces. Zeitschr. f. Kinderh., 1921, xxix, 253.

(187) Schittenhelm, A. Zur Frage der Ammoniakausscheidung im menschlichen Urin. Deutsch. Arch. f. klin. Med., 1903, lxxvii, 517.

(188) Schloss, E. Untersuchungen über den Einfluss der Salze auf den Säuglingsorganismus. Jahrb. f. Kinderh., 1910, lxxi, 296.

(189) Schloss, E. Fortschritte auf dem Gebiete des Mineralstoffwechsels im Säuglingsalter während der letzten drei Jahre. Jahrb. f. Kinderh., 1911, lxxiv, 91.

(190) Schlossmann, A. Die Reaktion des Säuglingsstuhle und ihre Bedeutung für die Praxis. Centralbl. f. Kinderh., 1906, xi, 237.

(191) Schlossmann, A. and H. Murschhauser. Der Stoffwechsel des Säuglings im Hunger. I–II. Biochem. Zeitschr., 1913, lvi, 355; 1914, lviii, 483.

(192) Schwarz, H. Der Stickstoff- und Schwefelstoffwechsel in Fällen von rachitischen Zwergwuchs und ein Beitrag zum normalen Stoffwechsel eines fünf Jahre alten Knaben. Jahrb. f. Kinderh., 1910, lxxii, 549; 712.

(193) Secchi, R. Über die Wirkung der Salzsäure auf die alkaliausscheidung. Biochem. Zeitschr., 1914, lxvii, 143.

(194) Sellards, A. W. The determination of the equilibrium in the human body between acids and bases with especial reference to acidosis and neuropathies. Johns Hopkins Hosp. Bull., 1912, xxiii, 289.

(195) Sellards, A. W. Tolerance for alkalies in Asiatic cholera. Philippine Journ. Sci., 1910, B., v, 363.

(196) Severin, L. Über die Wirkung Na_2CO_3 auf den Gehalt des Harns an Harnsäure and freie Säure. Marburg, 1868. Diss.

(197) Sherman, H. C. Bull. 185, U. S. Off. Exper. Sta. Cited in (198).

(198) Sherman, H. C., A. J. Mettler and J. E. Sinclair. Calcium, magnesium, and phosphorus in food and nutrition. U. S. Dep. Agric., Off. Exper. Stations. Bull., Wash., 1910, ccvii, 70 pp.

(199) Sherman, H. C. Chemistry of food and nutrition. 2nd ed. New York, 1918.

(200) Sherman, H. C. and J. E. Sinclair. The balance of acid-forming and base-forming elements in foods. Journ. Biol. Chem., 1907, iii, 307.

(201) Sherman, H. C. Phosphorus requirement of maintenance in man. Journ. Biol. Chem., 1920, xli, 173.

(202) Sherman, H. C. and A. O. Gettler. The balance of acid-forming elements in foods and its relation to ammonia metabolism. Journ. Biol. Chem., 1912, xi, 323.

(203) Sherman, H. C. and E. Hawley. Calcium and phosphorus metabolism in childhood. Journ. Biol. Chem., 1922, liii, 375.

(204) Sherman, H. C. Calcium requirement of maintenance in man. Journ. Biol. Chem., 1920, xlv, 21.

(205) Shohl, A. T. and A. Sato. Acid base metabolism. (To appear.)

(206) Söldner. Die Aschenbestandteile des neugeborenen Menschen und der Frauenmilch. Zeitschr. f. Biol., 1903, xliv, 61.

(207) Sommerfeld, P. Zur Kenntnis der chemischen Zusammensetzung des kindlichen Körpers im ersten Lebensjahre. Arch. f. Kinderh., 1900, xxx, 253.

(208) Spiro, K. Beiträge zur Lehre von der Säurenvergiftung bei Hund und Kaninchen. Beitr. z. chem. Phys. u. Path., 1902, i, 269.

(209) Stadelmann, E. Über die Ursachen der pathologischen Ammoniakausscheidung beim Diabetes mellitus und des Coma diabeticum. Arch. f. exper. Path. u. Pharm., 1888, xvii, 419.

(210) Stadelmann, E. Ueber den Einfluss der Alkalien auf den menschlichen Stoffwechsel. Experimentelle-klinische Untersuchungen. Stuttgart, 1890.

(211) Stehle, R. L. A study of the effect of hydrochloric acid on the mineral excretion of dogs. Journ. Biol. Chem., 1917, xxxi, 461.

(212) Stehle, R. L. and A. C. McCarty. The effects of hydrochloric acid ingestion upon the composition of the urine in man. Journ. Biol. Chem., 1921, xlvii, 315.

(213) Steinitz, F. Zur Kenntnis der chronischen Ernährungsstörungen der Säuglinge. I. Mitt.: Alkalistoffwechsel. Monatschr. f. Kinderh., 1902, i, 225.

(214) Steinitz, F. Zur Kenntnis der chronischen Ernährungsstörungen der Säuglinge. Jahrb. f. Kinderh., 1903, lvii, 689.

(215) Steinitz, F. Über den Einfluss von Ernährungsstörungen auf die chemische Zusammensetzung des Säuglingskörpers. Jahrb. f. Kinderh., 1904, lix, 447.

(216) Steinitz, F. and R. Weigert. Über die chemische Zusammensetzung eines ein Jahr alten atrophischen und rachitischen Kindes. Monatschrift f. Kinderh., 1905, iv, 301.

(217) Stütz. Alcalien, die wirksamsten, aber bisher grossentheils übersehenen Heilmittel in verschiednen Krankheiten des menschlichen Körpers. J. d. pract. Arznk. u. Wundarznk., 1800, x, 4. St., 3.

(218) Takeno, J. Beiträge zur Kenntnis des Stoffwechsels besonders der Mineralien im Säuglingsalter. II. Die Ausscheidung der wichtigsten organischen und anorganischen Nahrungsbestandteile im Kot unter wechselnden Ernährungs-Bedingungen. Jahrb. f. Kinderh., 1913, lxxvii, 640.

(219) Talbot, F. B. and L. W. Hill. The influence of lactose on the metabolism of an infant. Amer. Journ. Dis. Child., 1914, viii, 218.

(220) Taylor, A. E. Studies on an ash free diet. Univ. Calif. Pub., Path., 1903–04, i, 71.
(221) Tigerstedt, R. Zur Kenntnis der Aschenbestandteile in der frei gewählten Kost des Menschen. Skand. Arch. f. Physiol., 1910, xxiv, 97.
(222) Tobler, L. Über Veränderungen im Mineralstoffbestand des Säuglingskörpers bei akuten und chronischen Gewichtsverlusten. Jahrb. f. Kinderh., 1911, lxxiii, 566.
(223) Tobler, L. Zur Kenntnis der Chemismus akuter Gewichtsstürze. Arch. f. exper. Path. u. Pharm., 1910, lxii, 431.
(224) Tobler, L. and F. Noll. Zur Kenntnis der Mineralstoffwechsels beim gesunden Brustkind. Monatschr. f. Kinderh., 1910, ix, 210.
(225) Van Slyke, D. D. Studies of acidosis. X. Journ. Biol. Chem., 1918, xxxiii, 271.
(226) Van Slyke, D. D. and W. W. Palmer. Studies on acidosis. XVI. The titration of organic acids in urine. Journ. Biol. Chem., 1920, xli, 567.
(227) von Voit, C. Physiologie des allgemeinen Stoffwechsels und der Ernährung. In: Handb. d. Physiol., bearb. von H. Aubert et al., Leipzig, 1879–82, vi, Abt. 1.
(228) Voit, E. Ueber die Bedeutung des Kalkes für den thierischen Organismus. Zeitschr. f. Biol., 1880, xvi, 55.
(229) Wagner, C. Experimenta de experimento calcariae et magnesiae. Dorpati Liv., 1855. Diss.
(230) Walter, F. Untersuchungen über die Wirkung der Säuren auf den thierischen Organismus. Arch. f. exper. Path. u. Pharm., 1877, vii, 148.
(231) Weber, S. Ueber die Beeinflussung des Stoffwechsels durch einige pharmakologisch wichtige Stoffe. Ergbn. d. Physiol., 1904, iii, 233.
(232) Weigert, R. Über den Einfluss der Ernährung auf die chemische Zusammensetzung des Organismus. Jahrb. f. Kinderh., 1905, lxi, 178.
(233) Weiske, H. Ueber den Einfluss von Kalk- oder phosphorsäurearmer Nahrung auf die Zusammensetzung der Knochen. Zeitschr. f. Biol., 1871, vii, 178, 333.
(234) von Wendt, G. Untersuchungen über den Eiweiss- und Salz-Stoffwechsel beim Menschen. Skand. Arch. f. Physiol., 1905, xvii, 211.
(235) Wichura, S. De alcalium usu medico. Halae, 1801. Diss.
(236) Wilde, P. Disquisitiones quaedam de alcalibus per urinam excretis. Dorpat, 1855. Diss.
(237) Wilson, D. W. Acid-base equilibrium. (To appear.)
(238) Winterberg, H. Zur Theorie der Säurevergiftung. Zeitschr. f. physiol. Chem., 1898, xxv, 202.
(239) Wöhler, F. Versuche über den Übergang von Materien in den Harn. Zeitschr. f. Physiol., 1824, i, 125, 290.
(240) Wollf, G. Über den Kalk- und Phosphorsäurenstoffwechsel bei knapper und reichlicher Ernährung mit Kuhmilch. Jahrb. f. Kinderh., 1912, lxxvi, 180.
(241) Würtz, A. Versuche über die Verteilung der Phosphorsäure auf Harn und Kot. Biochem. Zeitschr., 1912, xlvi, 103.
(242) Ylppö, A. Neugeborenen-, Hunger- und Intoxikationsacidosis in ihren Beziehungen zu einander. Zeitschr. f. Kinderh., 1916, xiv, 268.

PURINE METABOLISM[1]

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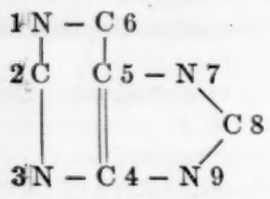
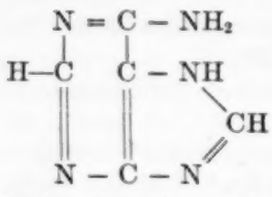
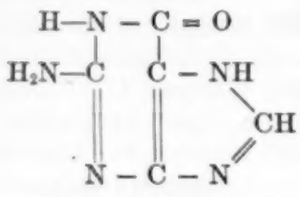
1. PURINES OF PHYSIOLOGICAL INTEREST. Of the many theoretically possible purines, the student of metabolism is chiefly concerned with five, namely, adenine, guanine, hypoxanthine, xanthine and uric acid. As shown by Emil Fischer (74), purines are all derivatives of a common "nucleus" or ring. The structural formulas for this ring, and for its five physiologically interesting derivatives, are indicated below.



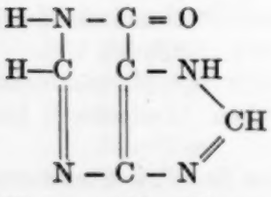
Purine "Nucleus"



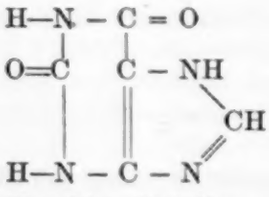
Adenine (6-amino-purine)



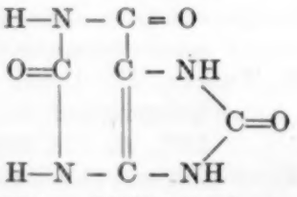
Guanine (2-amino-6 oxy-purine)



Hypoxanthine (6-oxy-purine)



Xanthine (2-6-dioxy-purine)



Uric acid (2-6-8-trioxy-purine)

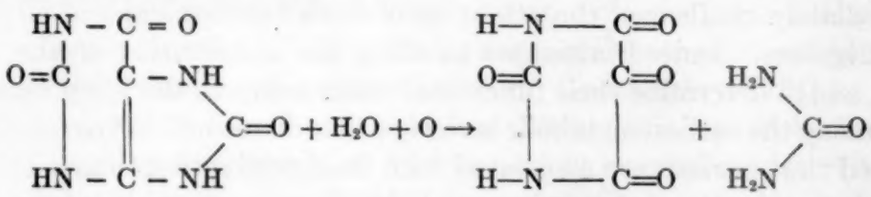
In addition to the above, certain methylated purines enter into the human dietary as ingredients of beverages. Their physiological fate will be referred to later.

Our knowledge of purine chemistry dates back to 1776, when uric acid was discovered by Scheele (267) as an ingredient of human urine, and almost simultaneously but independently by Bergmann (37) as a component of bladder stones. The other purines, with the exception

[1] It has been impossible in the space available for this review to refer to all of the publications related to purine metabolism. We have, therefore, confined our attention to those papers which in our opinion are most important, and which have served to introduce new ideas into the present-day conception of the subject.

of adenine, were discovered during the first half of the nineteenth century. Marcet (213) separated xanthine from urinary calculi. Guanine, first isolated from guano by Unger (310), was later reported by Virchow (311), (312) as an ingredient of concretions in swine. Hypoxanthine was discovered in heart and spleen tissue by Scherer (268), and later in the muscles of man and the lower animals by Strecker (289). Adenine, the last of the physiologically important purines, was not known until 1885, when Kossel (157), (158), (159), (160) succeeded in isolating it from pancreatic tissue. It remained for Emil Fischer (70), (75), (71), to show the relationship of adenine, guanine, hypoxanthine and xanthine to each other and to uric acid, and to devise methods for their synthesis (72), (73). It is beyond the scope of this article to present in detail the evidence upon which the present conception of the structure of the purines is based. It is sufficient to state that their structural relationship to uric acid has been abundantly established, and that the essential correctness of the uric acid formula is affirmed by the following facts:

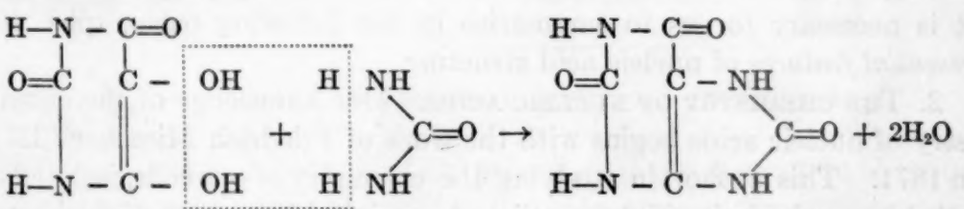
1. By the oxidation of uric acid with HNO_3 Liebig and Wöhler (204) obtained alloxan and urea.



Alloxan

This observation, together with a study of other oxidative disintegrations of uric acid, led Medicus (218) to suggest its correct formula.

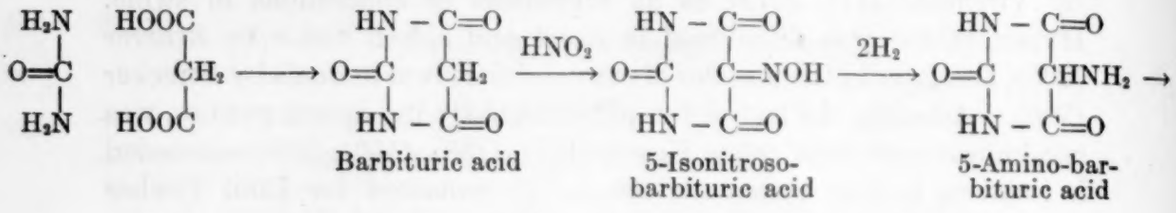
2. In 1888 Behrend and Roosen (29) accomplished the synthesis of uric acid by warming a mixture of 4-5-dihydroxy uracile and urea with concentrated H_2SO_4.

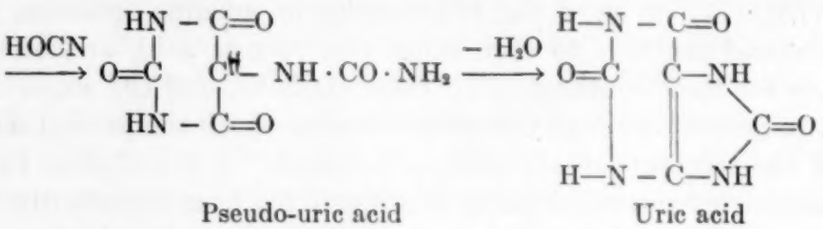


4-5-Dihydroxy uracile

3. In 1897 Fischer (72) showed that pseudo-uric acid, previously prepared from malonic acid by Baeyer (20), (21), is readily transformed

into uric acid by boiling with dilute mineral acids. This provided not only a complete synthesis of uric acid from malonic acid and urea, but removed the last vestige of doubt as to uric acid structure.





For detailed evidence concerning the structure of the other purines the reader is referred to the masterly studies of Emil Fischer (74).

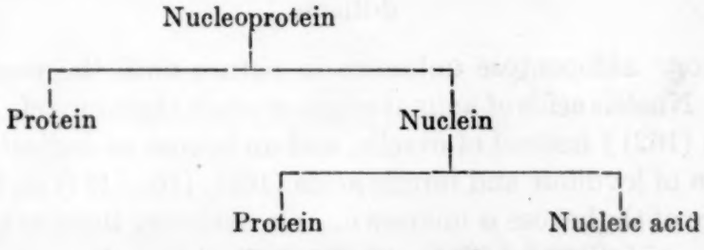
The discovery of the purines as ingredients of physiological materials immediately challenged the attention of both biochemical and clinical investigators. Indeed, attempts to study the distribution of the purines, and to determine their functional relationship to the life processes are among the earliest metabolic investigations on record. Progress soon showed that purines are associated with the nuclei rather than with the cytoplasm of cells, and this in turn led ultimately to the discovery of nucleic acids. It is not our purpose in an article of this scope to enter into a detailed discussion of the chemistry of nucleic acids. Such a discussion would be entirely superfluous in view of the availability of the excellent review of the subject by Jones (130). But inasmuch as the progress made in recent years in the study of purine metabolism has in large measure been the outgrowth of the discovery of nucleic acids, it is necessary for us to summarize in the following paragraphs the *essential features* of nucleic acid structure.

2. THE CHEMISTRY OF NUCLEIC ACIDS. Our knowledge of the chemistry of nucleic acids begins with the work of Friedrich Miescher (227) in 1871. This author, in studying the chemistry of pus cells, isolated a substance soluble in dilute alkali and precipitable with acetic acid, to which he gave the name of nuclein. Nuclein was found to contain phosphoric acid, and to respond to the protein color tests. Later Miescher (228) isolated a similar substance from the spermatic fluid of the salmon.

Nucleins were soon found to be characteristic of all cell nuclei. Hoppe-Seyler (109) isolated a similar product from yeast, and Kossel (154) another from the erythrocytes of birds. In his work upon the nuclein of salmon, Miescher discovered that the protein portion could be completely removed, leaving a protein-free acid. A similar acid prepared from yeast nuclein was later named "nucleic acid" by Altmann (13). In 1894 Kossel and Neumann (163) devised a method for the preparation of the nucleic acid of the thymus gland, and Neumann (243) showed that such acids can be isolated from a large number of nuclear materials.

Miescher did not recognize the relationship of nucleic acid to purines, or that the latter are important ingredients of the former. It remained for Kossel to prove that on hydrolysis nucleins liberate "alloxuric bases" or purines. He detected successively hypoxanthine (152), xanthine (153), guanine (155), (156), and finally adenine (159), (160). The oxypurines are now known to arise only as secondary products, and not to occur preformed in the nucleic acids. The discovery of the purines immediately suggested an origin for urinary uric acid, which before this time had been regarded as a product of ordinary protein metabolism.

Nucleic acids occur in the nuclei in combination with protein, probably a salt-like union, in which the protein plays the part of a base and is present in excess. These combinations constitute what are known as nucleo-proteins (the α-nucleoproteins of Hammarsten (101)). Some writers, notably Kossel and Lilienfeld (206), (205), are inclined to the belief that each molecule of nucleic acid is in combination with two molecules of protein, one of which is more readily removed by hydrolysis than is the other. According to this conception, nucleoproteins have the following structure:



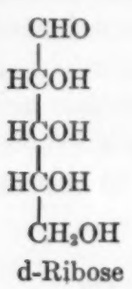
On the other hand, Jones is of the opinion that the proportion of protein depends largely upon the method of preparation of the nucleoprotein, and that the "terms nucleoprotein, nuclein and nucleic acid express a relation which means little more than that conveyed by the terms basic lead acetate, lead acetate and acetic acid" (130).

The study of nucleic acids has resulted in an accumulation of evidence indicating that there are only two such compounds in nature, one in the animal cell and typified by thymus nucleic acid, and the other in the plant cell and illustrated by yeast nucleic acid. Despite the fact that numerous tissues of both animal and plant origin have been analyzed, only nucleic acids of these two kinds have been discovered. They differ from each other in their ultimate hydrolytic products as illustrated below.

Products of hydrolysis of nucleic acids

Of animal origin	*Of plant origin*
Phosphoric acid	Phosphoric acid
Adenine	Adenine
Guanine	Guanine
Cytosine	Cytosine
Thymine	Uracile
Levulinic and formic acids	Pentose

Plant nucleic acids are characterized by containing the pyrimidine uracile, discovered by Ascoli (17), and of a pentose. The presence of the latter was proven by Kossel (161). It was identified by Levene and Jacobs (181), (183), (184), (188), (190) as d-ribose, a



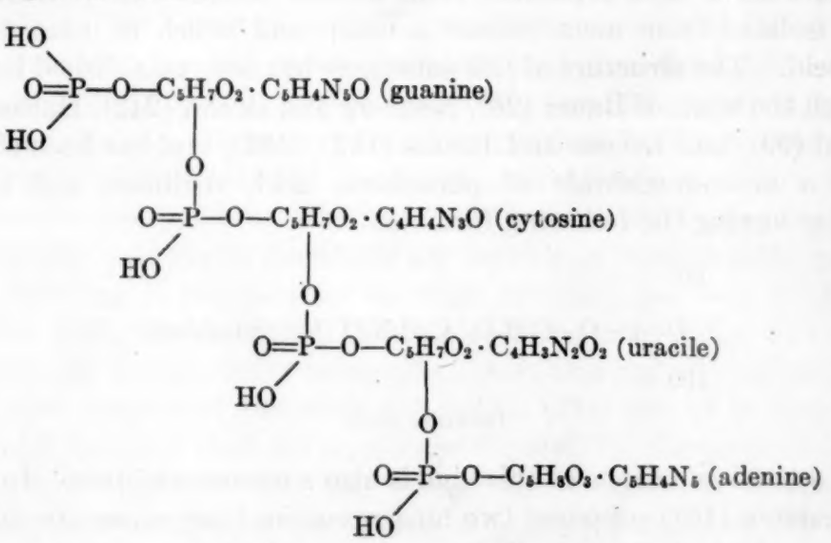
d-Ribose

levo-rotatory aldopentose unknown in nature until the work of these authors. Nucleic acids of animal origin contain thymine (cf. Kossel and Neumann (162)) instead of uracile, and an hexose as indicated by the production of levulinic and formic acids (163), (16), (121) on hydrolysis. The nature of the hexose is unknown. On oxidizing thymus nucleic acid with nitric acid, Steudel (284), (286), (287) obtained episaccharic acid ($C_6H_{10}O_8$). More recently Feulgen (62), (63) has suggested that the carbohydrate group may be glucal ($C_6H_{10}O_4$) instead of an hexose.

We are chiefly indebted to Levene and Jones for the information which we have concerning the union of the various hydrolytic products to form the complex nucleic acids. Levene and Jacobs (186), (187),

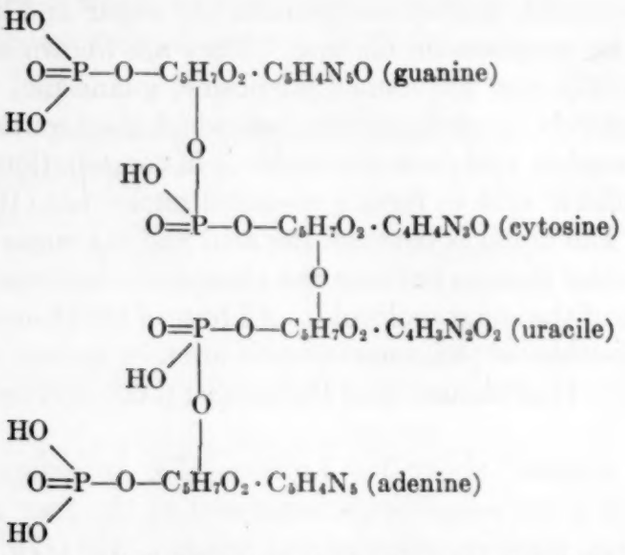
(189) found that neutral hydrolysis of yeast nucleic acid at 175°C. under pressure led to the separation of four crystalline compounds, each composed of a purine or pyrimidine molecule in combination with a molecule of d-ribose. The fact that these substances do not reduce alkaline copper solutions indicates that, at least in the case of the readily hydrolyzable purine compounds, the sugar and base are probably united by a glucoside binding. They are known collectively as nucleosides (195), and are named adenosine, guanosine, cytidine, and uridine respectively, according to the base which they contain. Furthermore, in the nucleic acid each nucleoside is in combination with a molecule of phosphoric acid to form a so-called nucleotide (195), or mononucleotide. The union is between the acid and the sugar (195), and is probably an ester linkage between the phosphoric acid and the primary alcohol group of the sugar molecule. All four of the theoretically possible mononucleotides of yeast nucleic acid are now known (cf. Jones and Kennedy (135), Thannhauser and Dorfmüller (300), Levene (177), (178), (179)).

The facts outlined above led Levene (175) to believe that yeast nucleic acid is a tetra-nucleotide composed of the four mono-nucleotides of adenine, guanine, cytosine and uracile. He (176) has recently suggested the following formula in which the nucleotides are represented as united to each other by ester linkages. Levene (180) is also of the opinion that thymus nucleic acid has an analogous structure, in which the four hexose nucleotides are combined through the phosphoric acid and sugar molecules.



Levene's formula for Yeast Nucleic Acid

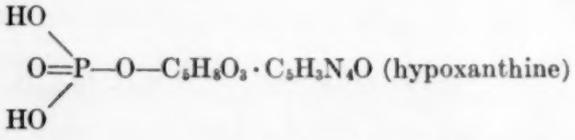
Until recently Jones (131) has advocated an ether linkage between the sugar molecules of the nucleotides. This idea has been accepted by Thannhauser and Sachs (301). Within the past few months Jones (137) has modified his views, and now represents the structure of yeast nucleic acid as follows:



Jones and Perkins' formula for Yeast Nucleic Acid

As to evidence for and against each of these formulas, the reader is referred to the original articles.

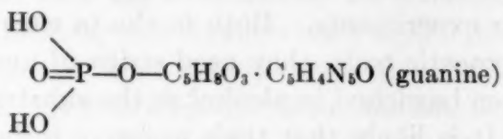
In addition to the true nucleic acids, two compounds of analogous structure have been separated from certain tissues. In 1847 Liebig (203) isolated from meat extract a compound which he named inosinic acid. The structure of this substance has been established largely through the work of Bauer (26), Neuberg and Brahn (242), Haiser and Wenzel (99), and Levene and Jacobs (182), (183), and has been shown to be a mono-nucleotide of phosphoric acid, d-ribose, and hypoxanthine having the following formula:



Inosinic Acid

The second substance of this kind is also a mononucleotide. In 1894 Hammarsten (101) prepared two nucleoproteins from pancreatic tissue, from one of which (his so-called β-nucleoprotein) he separated guanylic

acid by hydrolysis. Bang (23), (24), Steudel (285), and Levene and Jacobs (185), (192) have shown that guanylic acid has the following structure:



Guanylic Acid

Guanylic acid has also been isolated from a variety of glandular tissues other than the pancreas (cf. Jones and Rowntree (139), and Levene and Mandel (194)). Inosinic and guanylic acids are of peculiar interest in that they contain the sugar (d-ribose) characteristic of plant nucleic acids, and therefore cannot have their origin in the tetra-nucleotide of animal cells. They doubtless account for the wide distribution of pentose in animal tissues. There is no evidence to indicate that they occur in cell nuclei, but on the contrary, they probably exist in combination with the protein of the cytoplasm.

The discovery and identification of nucleic acids, and of the extranuclear inosinic and guanylic acids, served to greatly stimulate investigation of the physiological history of uric acid, the study of which had formerly been pursued in a more or less haphazard manner.

3. The digestion of exogenous nucleic acids (nucleoproteins). Abundant evidence has been accumulated indicating that the protein portions of ingested nucleoproteins are split off by the gastric and pancreatic juices with the liberation of free nucleic acids. In 1892 Lilienfeld (205) found that nucleoproteins are converted into proteins and "nuclein" by the gastric secretion, and that the nuclein is subsequently disintegrated by the pancreatic juice into protein and free nucleic acid. On the basis of these observations, and at the suggestion of Kossel, he proposed the scheme of nucleoprotein structure already alluded to (see p. 547). The fact that the proteolytic enzymes of the gastric and pancreatic secretions are capable of removing the protein, and digesting it just as they do other proteins, has been verified by Popoff (252), Umber (307), Harding and MacLean (103), and others. Apparently trypsin is far more effective in this regard than is pepsin.

In this connection the work of Schmidt (274) should be mentioned. Schmidt believed that cell nuclei are digested by the pancreatic juice only, and suggested that the microscopic examination of the stools for cell nuclei following the feeding of nuclear material might be employed as a diagnostic test for inefficiency in pancreatic digestion. Sev-

eral investigators, notably Strauch (288), were ardent defenders of the alleged value of the "Schmidt test." It is likely that both Schmidt and Strauch were deceived by the slowness of peptic digestion under the conditions of their experiments. Both in the *in vitro* experiments and in the clinical diagnostic tests, they used strips of muscle tissue which had previously been hardened in alcohol as the substrate for the action of the enzymes. It is likely that their negative tests are attributable to the relative indigestibility of the alcohol-hardened tissue. That pepsin can slowly digest cell nuclei, even after hardening of the tissue, has been shown by Hesse (107) and Kashiwado (141). The results of the latter investigators, and of van Westenrijk (323), indicate that the Schmidt test is unreliable. Certainly there seems to be no occasion for doubting that the alimentary enzymes, under ordinary conditions, completely digest the protein portion of the nucleoprotein.

The gastric and pancreatic juices are generally believed to be entirely without influence upon nucleic acids. Milroy (230) believed that trypsin slowly removes phosphoric acid in some organic form from nucleoproteins, and Abderhalden and Kashiwado (4) report that part of the phosphoric acid is split off under the influence of the gastric and pancreatic secretions. That such reactions could occur to an appreciable extent seems quite unlikely in view of the investigations of others. Abderhalden and Schittenhelm (5) had previously reported that pancreatic juice collected from fistula animals, either with or without the addition of enterokinase, decomposes thymus nucleic acid in such a way as to destroy its power of gelatinizing, but without the liberation of free purines. They also stated that gastric juice was without effect upon nucleic acid. Levene and Medigreceanu (197) were unable to detect an enzyme capable of decomposing nucleic acids in either the gastric or pancreatic secretions. Furthermore, the early experiments of Milroy are not convincing. This author employed extracts of the pancreas for his experiments, instead of digestive juices collected from fistula animals. It is conceivable that such enzyme preparations may have contained intracellular purine ferments. As we shall see later, intracellular enzymes produce more profound disintegrative changes in the nucleic acid molecule than do alimentary secretions. For the most part Milroy's experiments were made with artificial nucleins prepared by bringing together faintly acid solutions of proteins and nucleic acid. Jones (127) has shown that trypsin is without effect upon the nucleic acid part of nucleoproteins when the latter are purified, and dried by washing with alcohol and ether. Under these conditions there is no liberation of

phosphoric acid. If, however, the nucleoprotein is not so purified and dried, intracellular enzymes from the tissue used in the preparation of the substrate apparently are adsorbed by the nucleoprotein, in spite of frequent reprecipitations, and through their action may lead to erroneous conclusions. Jones' work clearly shows the complete independence of trypsin and the enzyme responsible for nucleic acid disintegration. Other investigators (cf. Biondi (40), Iwanoff (122) and Sachs (265)) also recognized the latter fact, but the experimental data upon which their opinions were based are not as convincing as those of Jones.

Nakayama (240) found an increase in free phosphoric acid when extracts of intestinal mucosa were permitted to act upon nucleic acid. He made the rather remarkable deduction that the enzyme responsible for the splitting of nucleic acid is identical with erepsin. More likely, as pointed out by Jones (130, p. 60), the experiments of Nakayama merely show the similarity in distribution of the two enzymes, and indicate that it is difficult to obtain an erepsin preparation devoid of the enzyme concerned in nucleic acid disintegration. It is quite unlikely that a member of a group of substances which are characterized by such remarkable specificity in action as are enzymes should act upon two substrates so widely different as peptones and nucleic acids.

The most careful and complete experiments reported concerning the action of alimentary enzymes upon nucleic acids are those of Levene and his associates. Using extracts of the intestinal mucosa and pure gastric, pancreatic and intestinal juices obtained from Pawlow fistula animals, Levene and Medigreceanu (197), (198) found by optical methods of study that three groups of enzymes are concerned in nucleic acid digestion, instead of a single ferment, "nuclease" (122), as had formerly been believed. They employed as substrates not only yeast and thymus nucleic acids, but a variety of fragments of the nucleic acid molecules, which differed widely from each other in structural complexity. Their investigations led to the following conclusions: *a*, Gastric and pancreatic juices are not concerned in nucleic acid disintegrations. *b*, Intestinal juice contains an enzyme (nucleinase) which hydrolyzes tetra-nucleotides into mono-nucleotides, and another enzyme or group of enzymes (nucleotidases) which hydrolyze purine nucleotides into phosphoric acid and nucleosides. The pyrimidine nucleotides are probably not affected by the *succus entericus*. *c*, Extracts of the intestinal mucosa contain nucleinase, nucleotidases for both the pyrimidine and purine nucleotides, and a third enzyme or group of enzymes (nucleosidases) capable of hydrolyzing the purine nucleosides into sugar and

bases. The pyrimidine nucleosides are apparently not hydrolyzed in the intestinal wall. Indeed, studies of Levene and La Forge (193) indicate that they are not hydrolyzed into sugar and bases by extracts of any tissue.

At about the time the work of Levene and Medigreceanu appeared, London and Schittenhelm (208), and London, Schittenhelm and Wiener (209), (210) published the results of feeding experiments in fistula dogs. By means of experiments in which nucleic acid was administered orally, and removed at various stages in its passage through the alimentary canal, the authors arrived at the following conclusions: *a*, The stomach does not digest or absorb nucleic acids. *b*, In the intestine, chemical changes occur in such a fashion as to produce small amount of free purines, but larger amounts of purine compounds of a diffusible nature (nucleotides and nucleosides?). *c*, The exclusion of pancreatic juice from the intestine, or even the extirpation of the stomach or the pancreas, is without effect upon nucleic acid digestion. *d*, The results of *in vitro* experiments agree with the *in vivo* data in showing that pancreatic juice exerts no action, while intestinal juice manifests marked digestive effect upon nucleic acid. It is thus seen that in their essential features, the conclusions of London and his associates are in accord with the findings of Levene and Medigreceanu. It is unfortunate that London, Schittenhelm and Wiener (209), (210) claim to have identified guanosine, and also to have detected a substance having the properties of guanylic acid, following the feeding of thymus nucleic acid. Since guanosine and guanylic acid are products of the disintegration of *yeast nucleic acid*, it is obviously impossible for them to have had their origin in *thymus nucleic acid*. Levene and Jacobs (191) have suggested as a possible explanation of these remarkable observations, that the thymus nucleic acid employed by London, Schittenhelm and Wiener may have been an impure product containing guanylic acid. Nevertheless, the fact that London and his colleagues report such unique findings without attempting to offer a satisfactory explanation for them must necessarily shake one's confidence somewhat in the validity of their other results.

Recently Thannhauser and Dorfmüller (295), (298) have reported that human duodenal fluid splits yeast nucleic acid into uracile nucleotide and a triphosphonucleic acid. It is very probable that their so-called triphosphonucleic acid is a mixture of mono-nucleotides (cf. Jones (130), p. 39). They find (299) that intestinal bacteria disintegrate the purine ring with the liberation of ammonia, and that this accounts for the failure to recover quantitatively purines (or nucleic acids) ad-

ministered *per os*. They believe that under ordinary conditions purines are absorbed probably as nucleosides, and in accord with this conception, they have been unable to detect free purines, other than uric acid, in the blood (297).

There seems to be very little doubt, therefore, that in the alimentary tract nucleic acids are progressively broken down through the intermediary stages of nucleotides and nucleosides. How far these reactions proceed is uncertain. At the completion of the action of the enzymes of the *succus entericus*, and of the intracellular enzymes in the intestinal wall, the products available for absorption are probably phosphoric acid and nucleosides, with more or less sugar (hexose or d-ribose), adenine, and guanine. Such information as we have indicates that all of these products enter the portal circulation. The investigations of Biberfeld and Schmid (39) show that purines are not absorbed via the lymphatics. In animals with thoracic fistulas, these authors were unable to detect the slightest increase in the free or combined purines of the lymph following the feeding of thymus nucleic acid. Indeed, detectable amounts of purines were never found even after hydrolysis of the lymph with 3 per cent sulphuric acid. We are, therefore, forced to assume for the present, that the ingredients of nucleic acids are absorbed by way of the portal circulation. The fate of the purines, with which this article is particularly concerned, will be considered below.

4. THE ANABOLISM OF PURINES. Comparatively little is known concerning the anabolism of purines. The animal organism is certainly not dependent upon preformed purines in the diet for the formation of nucleic acids in the cell nuclei. The observations of Miescher (228) in 1874, that the migrating salmon forms relatively enormous amounts of nuclein for the generation of spermatozoa, indirectly indicates the ability of this animal to synthesize purines. Numerous investigations have confirmed the findings of Miescher, and have established the fact that the salmon, during its long migration from the sea to the spawning grounds, abstains from food, and forms the nuclear material at the expense of its own muscle tissue.

Since the work of Miescher, the problem of purine synthesis has been attacked from several different points of view, and direct and convincing proof has been provided that the cells of both adult and young animals are capable of synthesizing purines to the fullest extent for anabolic purposes. The first to study the question was Tichomiroff (305), who, working with the ova of the silkworm, *Bombyx mori L.*, found almost a tenfold increase in purines during the embryonic development of the

eggs. Obviously, these purines must have had their origin in some non-purine ingredient of the ova. Soon after the work of Tichomiroff, Kossel (159) made similar experiments upon hens' eggs, and obtained like results. In spite of numerous attempts, Kossel was unable to detect purines in fresh eggs, but after incubating an egg for fifteen days isolated 8.4 mgm. of guanine and 19.5 mgm. of hypoxanthine (perhaps also adenine). Calculated on a basis of the dry substance in the embryo, the figures amounted to approximately 0.94 per cent of purines. Mendel and Leavenworth (221), using more refined methods, repeated and extended the experiments of Kossel, and obtained uniformly progressive increases in the purine content of both hens' and ducks' eggs during incubation. They succeeded also in isolating and identifying adenine, guanine and hypoxanthine, which are characteristic components of nucleoproteins. These experiments afforded conclusive proof of a synthesis of purines during embryonic development.

Corroboratory evidence of synthesis was supplied by Plimmer and Scott (251) in studies upon the distribution of the various forms of phosphorus in the egg during incubation. They report that the phosphoric acid of the nucleic acid probably has its origin in the vitellin of the yolk. In rats, McCollum (216) has shown that inorganic phosphates may serve as the sole supply of phosphorus for nuclein synthesis (cf. also Fingerling (69)).

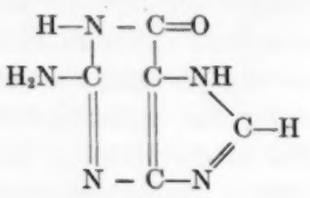
At a somewhat later date Burian and Schur (49) proved the synthesis of purines in growing mammals. Suckling rabbits and pups were permitted to grow for varying periods, and compared as regards their purine content with animals of the same litter which had been killed and analyzed at the beginning of the experiment. In two of the typical rabbit experiments, the increases in purine nitrogen amounted to 94.1 and 136.7 mgm. respectively during eighteen-day periods. The authors were unable to secure sufficient rabbit's milk for the determination of its purine content. Analyses of cow's milk showed the presence of 4.12 to 6.40 mgm. of purine nitrogen per liter. If rabbit's milk contains a similar quantity of purine nitrogen, it is obviously impossible for the increased purine content of the tissues to have had an exclusively exogenous origin. For such to have been the case would have necessitated the consumption of a liter or more of milk per day by each of the growing animals. Nor are the conclusions of Burian and Schur invalidated by the recent determinations of purines in cow's milk by Voegtlin and Sherwin, and Denis and Minot. These authors report that cow's milk contains 5 mgm. of adenine and 10 mgm. of guanine per

liter "as minimum values" (313), and about 15 mgm. of uric acid per liter (59), or a nitrogen content in the form of these three purines of 12.2 mgm. per liter. Even if we assume that the actual purine content of milk is 100 per cent greater than the combined values obtained by Voegtlin and Sherwin, and Denis and Minot, or 25 mgm. of purine nitrogen per liter, we would still be compelled to assume a milk consumption of 200 to 300 cc. per animal per day in order to account for the data of Burian and Schur.

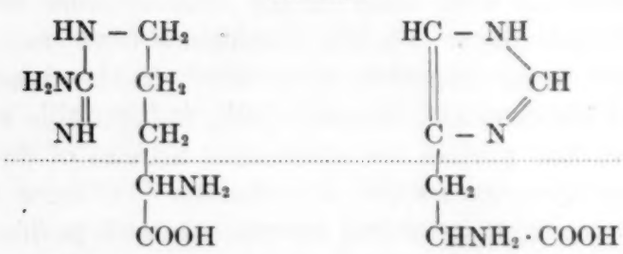
But fortunately we are not limited to evidence of this sort, in which there is the possibility of a marked difference in the purine content of cow's and rabbit's milk (cf. Denis and Talbot (60) for uric acid content of human milk) which might account for the experimental findings. Abundant evidence of an entirely different kind has been accumulated indicating that purines are not necessary articles of diet. As early as 1891 Socin (280), in studying the utilization of inorganic and organic iron compounds, succeeded in maintaining the health and growth of mice upon a purine-free diet of cooked egg-yolk, starch, cellulose, and water. Experiments with some of his animals were continued for sixty to ninety-nine days. Similar conclusions have been reached by McCollum (216) in investigations upon rats. In the elaborate growth experiments of Osborne and Mendel (249), indisputable evidence has been presented that purines are unessential articles of diet, and both they and other investigators (cf. Abderhalden (1)) have verified this fact over and over again in feeding experiments with purified foodstuffs. Moreover, Benedict (32) has made the remarkable observation that a Dalmatian dog, which normally eliminates uric acid in the urine, may be maintained upon a purine-free diet, and continue to eliminate uric acid. In one such experiment a Dalmatian was kept upon a diet devoid of purines for nearly a year, and during this time excreted more than 100 grams of uric acid. Not 10 per cent of this could have come from the preformed purines of the animal's tissues. Thus the power of purine synthesis it not limited to growing animals, but is characteristic of the adult mammal as well.

Finally, Kollmann (151) has recently reported experimental results indicative of purine synthesis in adult man. A healthy young woman was kept upon a constant diet of very low purine content for a period of 50 days. Despite the fact that she gained 4 kgm. in body weight during this time, the uric acid output exceeded the total purine intake by 15 grams for the experimental period. Evidently synthesis of the purine ring must have occurred.

As to the mechanism of purine formation, and the intermediary steps involved, the literature affords very little information. The field is almost entirely unexplored. Obviously, the synthesized purines must have their origin in one or more of the amino-acids of the protein molecule, but the only publication, with which we are familiar, that throws any light upon the problem is the interesting paper of Ackroyd and Hopkins (10). It occurred to these authors that purines might have their origin in arginine and histidine, the pyrimidine portion of the molecule coming from the former, and the imidazole ring from the latter. In this case both arginine and histidine would be necessary for purine synthesis, as indicated in the formulas below.



Guanine



Arginine Histidine

To test this hypothesis they compared the growth and allantoine output of rats upon diets in which the nitrogenous requirements were supplied respectively by completely hydrolyzed casein, and hydrolyzed casein from which arginine and histidine had been removed. Both types of diet were supplemented with cystine, tryptophane and vitamines in sufficient quantities to render them adequate in these respects for the nutritive requirements of the animals. Upon the ingestion of the diet devoid of the two amino-acids, the animals promptly and rapidly lost weight, and showed decreases of 40 to 50 per cent in the elimination of allantoine. Contrary to the authors' original expectations, however, the addition to the deficient diet of either of the missing amino-acids was followed by a resumption in growth, and an increase in the excretion of allantoine. Furthermore, the decrease in allantoine excretion was much less when either arginine or histidine alone

TABLE 1*

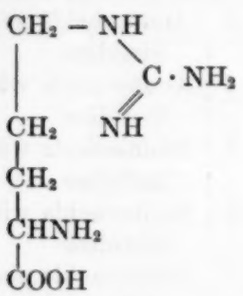
Effect of arginine and histidine upon allantoine excretion

WEEK	MEAN WEIGHT DURING WEEK		ALLANTOINE FROM TWO RATS PER WEEK	NITROGEN PER RAT PER DAY			ALLANTOINE N AS PER CENT TOTAL N	DIET
	Rat A	Rat B		Total N	Purine N	Allantoine N		
	Experiment I							
1914	*gms.*	*gms.*	*mgm.*	*mgm.*	*mgm.*	*mgm.*		
1	124	132	458	147	0.65	11.6	7.8	Bread and milk
2	116	126	296	95	0.44	8.0	8.4	Amino-acids without arginine and histidine
3	109	116	260	107	0.30	6.5	6.3	Amino-acids without arginine and histidine
4	105	109	319	116	0.40	8.0	6.7	Amino-acids without arginine and histidine
5	102	106	228	104	0.23	5.8	5.6	Amino-acids without arginine and histidine
6	113	114	347	134	0.36	9.0	6.7	Amino-acids plus arginine and histidine
7	121	124	347	133	0.34	9.0	6.7	Amino-acids plus arginine and histidine
8	131	131	400	157	0.49	10.1	7.0	Amino-acids plus arginine and histidine
9	137	141	454	199	0.69	11.5	5.8	Bread and milk
	Experiment II							
	Rat C	Rat D						
1	146	125	575	182	0.63	14.6	8.0	Bread and milk
2	139	119	406	105	0.44	10.3	9.8	Amino-acids without arginine and histidine
3	125	112	306	111	0.46	7.7	7.0	Amino-acids without arginine and histidine
4	119	106	390	142	0.37	9.9	7.0	Amino-acids without arginine and histidine
5	120	106	310	130	0.39	7.9	6.1	Amino-acids plus arginine and histidine
6	125	115	413	119	0.46	10.5	8.8	Amino-acids plus arginine and histidine
7	131	120	400	135	0.53	9.9	7.3	Amino-acids plus arginine and histidine
8	133	127	486	136	0.50	12.3	9.0	Amino-acids plus arginine and histidine
9	142	137	501	167	0.51	13.6	8.1	Bread and milk

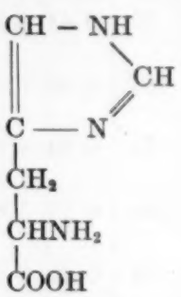
* Table taken from Ackroyd and Hopkins (10).

was removed from the ration, than when both were excluded. The results of two typical experiments of Ackroyd and Hopkins are reproduced in table 1. The figures for the daily elimination of total-, purine-, and allantoine-nitrogen represent the averages for two rats.

The experimental findings led Ackroyd and Hopkins to modify their original conception, and to conclude that arginine and histidine are replaceable the one by the other in nutrition,[2] and that each may serve as the raw material for the synthesis of the purines. The structural similarity of these two amino-acids is evident from the formulas below.



Arginine



Histidine

We shall consider the paper of Ackroyd and Hopkins in another connection later.

There is one other question in connection with purine anabolism which should be briefly considered, namely, the possibility of the utilization of *exogenous* purines for nuclein formation. We have already seen that purines are not necessary ingredients of the diet, but this fact does not exclude the possibility of their being anabolized into nucleic acids *when they are present*, in lieu of a synthesis of purines from the usual precursors. No conclusive answer to this question is to be found in the literature. In a recent paper the writer (262) tentatively suggested that in conditions of physiological stress, such as starvation, the purines liberated in tissue wear and tear might possibly be re-utilized for anabolic purposes. A similar possibility is presented in the case of exogenous purines. While it is exceedingly difficult to exclude such an

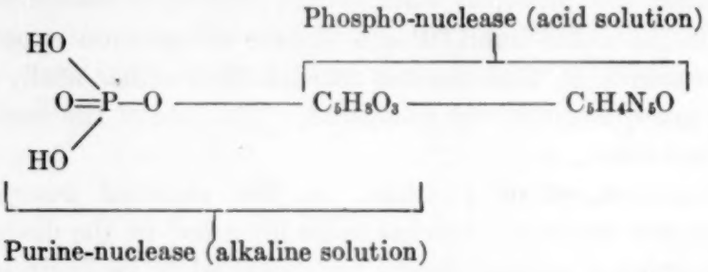
[2] Investigations in this laboratory have served to cast doubt upon the validity of Ackroyd and Hopkins' conclusions regarding the relation of arginine and histidine to growth. Our preliminary experiments indicate that these amino-acids cannot replace each other in nutrition (Rose and Cox (263)). However, our observations do not necessarily affect the deductions of Ackroyd and Hopkins concerning purine formation from the diamino-acids. We expect to present our data in full in the near future.

occurrence in the case of at least a part of the ready-formed purines of the diet, it must be admitted that the evidence available is probably unfavorable to such a view. The prompt increase in the elimination of uric acid or allantoine following the ingestion of nucleic acids or purines (cf. Krüger and Schmid (171), Schittenhelm and Bendix (270), Mendel and Brown (220), Mendel and Lyman (222)), would seem to indicate that the latter are promptly catabolized, and their end-products discarded. On the other hand, it is a matter of common experience, as will be discussed later, that purines administered either orally or parenterally are not quantitatively recovered. The fate of the uneliminated portion is unknown.

5. THE CATABOLISM OF PURINES. *a. The chemical transformations and the enzymes involved.* Nucleic acids liberated in the disintegration of nucleoproteins of animal tissues are believed to be hydrolyzed into their ultimate components in a manner quite similar to that which occurs in the case of exogenous nucleic acids during digestion in the alimentary tract. However, more profound decomposition is brought about under the influence of the intracellular enzymes than is occasioned by alimentary ferments, and the process is more complex inasmuch as a larger number of enzymes is involved, and a greater variety of transformations is thus made possible. In the catabolism of nucleic acids and the component purines, one sees a remarkable illustration of specificity in enzymatic reactions. Nowhere else in metabolism is the division of physiological labor between several catalyzers so beautifully exemplified as in the disintegration of nucleic acids.

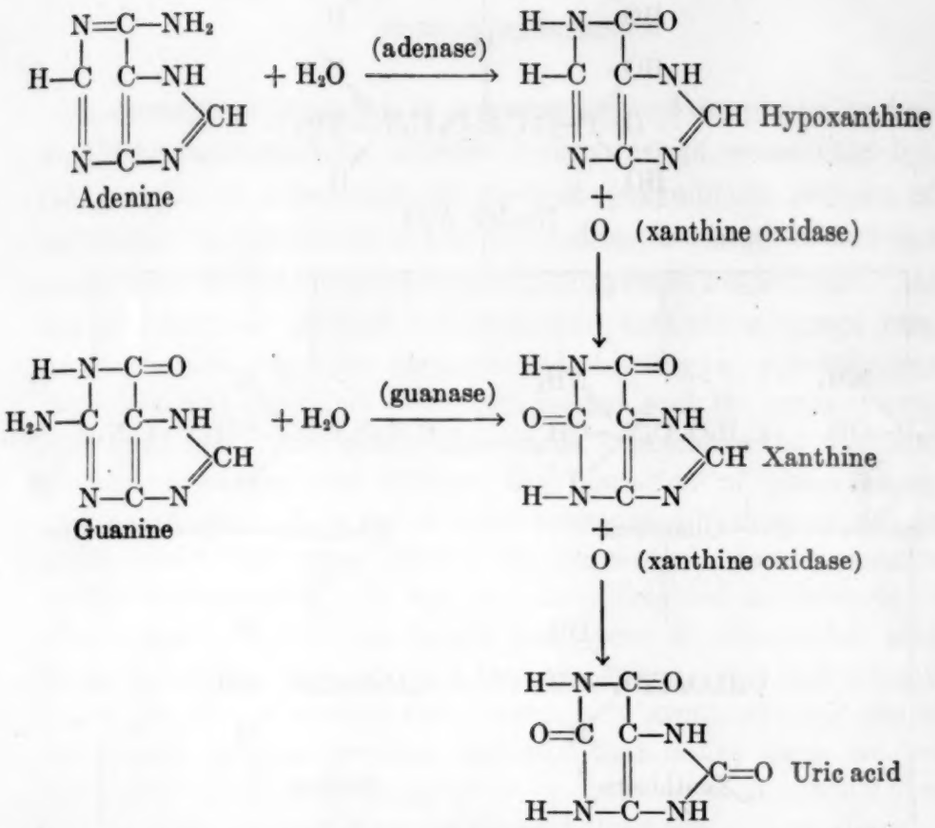
According to Levene and Medigreceanu (196), (198), practically all organs of the body, as far as they have been examined, contain nucleinases which hydrolyze poly-nucleotides into mono-nucleotides, nucleotidases which decompose mono-nucleotides into phosphoric acid and nucleosides, and nucleosidases which split nucleosides into their component sugar and base. Inasmuch as the pyrimidine nucleotides and nucleosides are hydrolyzed by ferments which are different from those which decompose the purine nucleotides and nucleosides, it seems quite probable that four specific ferments of each class are concerned in nucleic acid disintegration. But other paths of decomposition may also occur besides those proven by Levene and Medigreceanu. Leaving the pyrimidine nucleotides out of consideration, Jones (128), (129) has shown that the purine nucleotides may undergo enzymatic disintegration in two different ways. Phosphoric acid may be removed leaving the nucleosides, or the purine bases may be removed leaving

the sugar and phosphoric acid combined. Amberg and Jones (14) have named the first of these enzymes "phospho-nuclease" (nucleotidase of Levene and Medigreceanu), while they designate the second as "purine-nuclease." Phospho-nuclease acts most rapidly in a faintly acid solution, and purine-nuclease is most effective in a faintly alkaline solution (130).



Furthermore, Jones (129) and Amberg and Jones (14) have shown that deamination of the purines may occur before they have been liberated from the nucleosides. Thus adenosine may be transformed into inosine, and guanosine into xanthosine. The enzymes responsible for these changes are known as adenosine-deaminase (14), and guanosine-deaminase (129) respectively. Other ferments, which Jones and his associates call adenosine-hydrolase (15), guanosine-hydrolase (134), xanthosine-hydrolase (129), and inosine-hydrolase (14) respectively, hydrolyze the corresponding nucleosides into their component purines and sugar. Thus the path of nucleic acid decomposition may vary with conditions, and with the enzymes available in the organ or tissue in question.

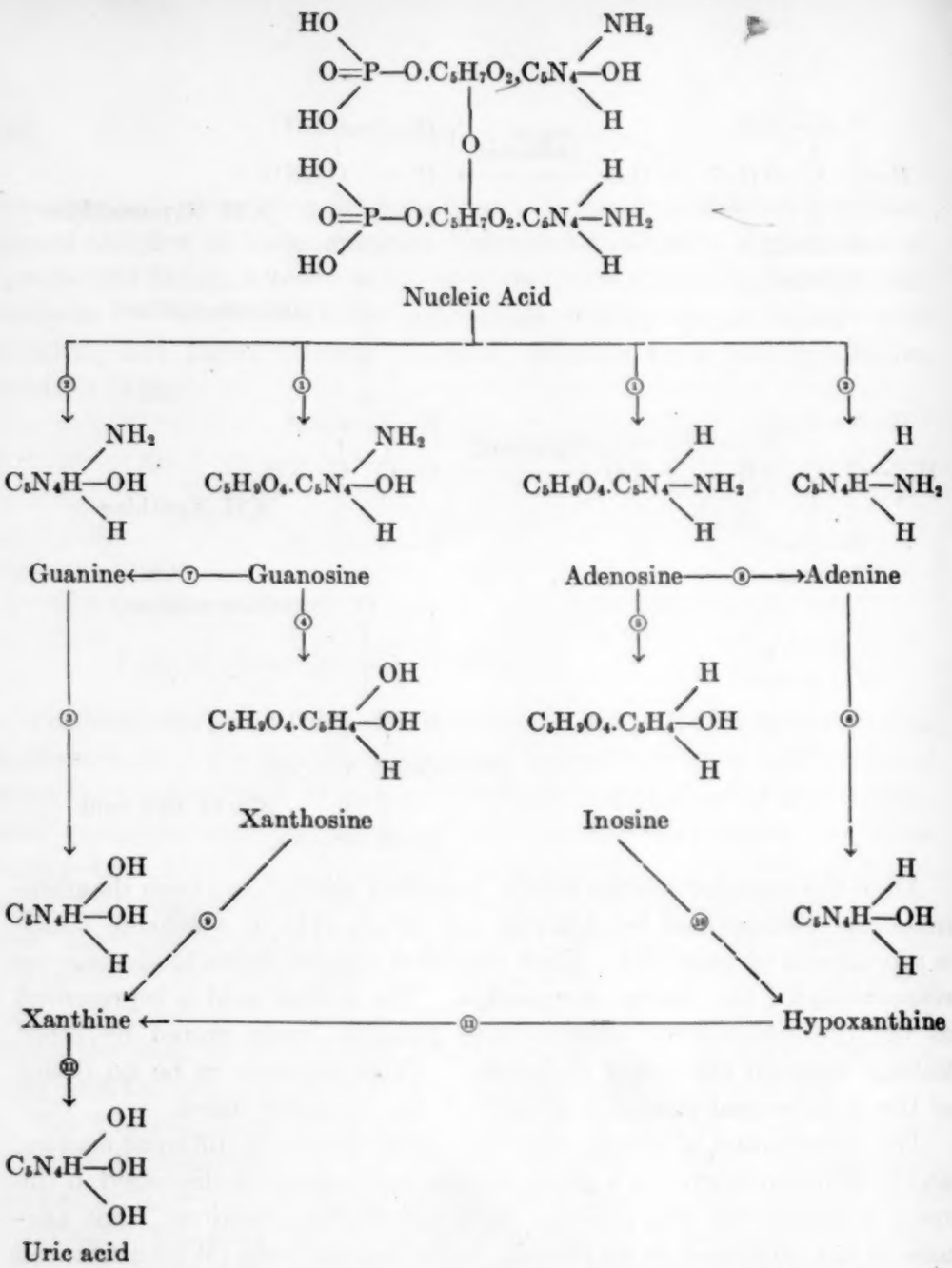
The purines liberated in these reactions, as well as those produced by the decomposition of the mono-nucleotides (inosinic and guanylic acids) of muscular, pancreatic, and other tissues, are believed to be oxidized to uric acid through the instrumentality of the enzymes adenase (140), guanase (136), and xanthine oxidase (282), (329), as indicated in the formulas below.



All of the transformations briefly described above have been diagrammatically summarized by Amberg and Jones (14) in a scheme which is reproduced on page 564. Each encircled number refers to the enzyme responsible for the change in question. The nucleic acid is represented as the di-nucleotide of adenylic and guanylic acids united by ether linkage through the sugar molecules. There appears to be no doubt of the independent existence of each of the ferments listed.

The distribution of purine enzymes varies widely in different species, and in different organs of a given species, and cannot be discussed in the space available for this article. It is noteworthy, however, that adenase is not supposed to be present in the human body (Winternitz and Jones (334)), with the possible exception of the mixed organs of embryos (Long (211)). It is probable, therefore, that under ordinary conditions in man adenine is deaminized before it is liberated from its nucleoside.[3] For further information concerning the occurrence of purine ferments, reference should be made to the monograph of Jones (130). A resumé of the literature will also be found in the publications of Bloch (41), (42), (43).

[3] An interesting paper by H. Jackson has just appeared (Journ. Biol. Chem., 1923, lvii, 121) in which the author presents evidence indicating the occurrence of *adenine nucleotide* in human blood. He believes that 15–25 mgm. per 100 cc. are normally present, and that this compound accounts for a considerable part of the undetermined nitrogen of the circulating fluid.



(1) Phospho-nuclease (Amberg and Jones (14)), or nucleotidase (Levene and Medigreceanu (198)).

(2) Purine-nuclease (Amberg and Jones (14)).

(3) Guanase (Jones and Partridge (136)).

(4) Guanosine-deaminase (Jones (129)).

(5) Adenosine-deaminase (Amberg and Jones (14)).

(6) Adenase (Jones and Winternitz (140)).

(7) Guanosine hydrolase (Jones and Belt (134)), or nucleosidase (Levene and Medigreceanu (198)).

(8) Adenosine hydrolase (Amberg and Jones (15)), or nucleosidase (Levene and Medigreceanu (198)).

(9) Xanthosine-hydrolase (Jones (129)).

(10) Inosine-hydrolase (Amberg and Jones (14)), or nucleosidase (Levene and Medigreceanu (198)).

(11) Xanthine oxidase (Spitzer (282), Wiener (329)).

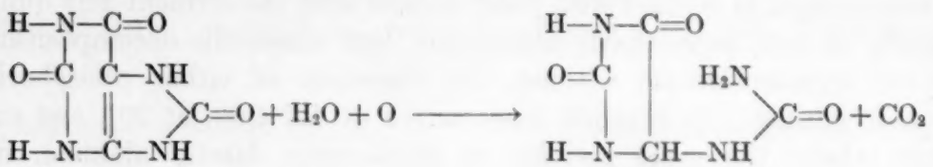
In connection with the enzymes concerned in purine metabolism, should be mentioned the interesting discovery of recent data by Jones (132), (133), of a thermostable catalyst (enzyme?) in extracts of pig's pancreas. The pancreas of the pig contains a variety of active agents which may be instrumental in the hydrolysis of nucleic acid. According to Jones, all of these are destroyed by boiling except one, viz., the one which acts upon the nucleotide bindings of yeast nucleic acid. When brought in contact with yeast nucleic acid, the ferment acts quite rapidly at first, more slowly afterwards, but causes the decomposition of the tetra-nucleotide without the liberation of either phosphoric acid or purines. It is much more active at 40° than at 20°, and exhibits about the same activity in amphoteric, faintly alkaline, or faintly acid reaction. It has not been detected in extracts of any other organ or tissue, nor does it decompose thymus nucleic acid. As stated by Jones (133), the fact that the spleen, liver and other tissues of the pig do not contain the thermostable agent, and yet are able to disintegrate poly-nucleotides, indicates that either there are two ferments, one of which is thermolabile, or that the decomposition of nucleic acid by tissue extracts does not proceed along conventional lines.

At first Jones believed that the heat-stable enzyme completely disintegrates the nucleic acid into its four component nucleotides. More recently Jones and Perkins (137) have found that this is not the case. They state that the active agent acts upon nucleotide linkages only, but that it does not split the nucleic acid into mono-nucleotides, since "the end product contains intermediate substances between nucleic acid and mono-nucleotides." The rupture produces no increase in titratable acidity, in contrast to the effect of 1 per cent sodium hydroxide, which at room temperature also decomposes nucleic acid, but with appreciable increase in titratable acidity (Jones and Perkins (138)). This difference in behavior of nucleic acid under the influence of the two agents, thermostable enzyme and 1 per cent alkali, indicates, according to Jones, that at least one of the nucleotide linkages of yeast nucleic acid is through the carbohydrate molecules, and has led him to suggest his latest formula for nucleic acid, which is given above (p. 550).

It is impossible at the present time to offer any explanation for the presence of the thermostable agent. Future investigations of Jones along this line will be followed with great interest. Catalyzers resistant to heat have not often been reported in the animal body. Hammett (102) has recently found in muscle tissue a thermostable agent capable of transforming creatine into creatinine, an observation which we have

been able to verify in this laboratory (264). Perhaps future investigations will indicate that such catalysts are not as rare as our present lack of knowledge concerning them would seem to indicate.

b. The physiological oxidation of uric acid (uricolysis). In most mammals the uric acid arising in purine catabolism by reactions outlined above is not eliminated as such, but undergoes further oxidation, probably with the production of allantoine.



Allantoine

Evidence of the occurrence of this reaction was presented by Salkowski (266), who observed the elimination of allantoine in dogs following the administration of uric acid. Corroboration of his findings was provided by a number of investigators, notably Cohn (53), Minkowski (232), Mendel and Brown (219), Mendel and White (225), and Wiechowski (325). Underhill and Kleiner (309) first showed that the urine of fasting dogs contains allantoine, thus proving that it is not derived exclusively from dietary purines. Furthermore, extracts of tissues have been shown to possess the power of uric acid destruction (cf. Brunton (47), Wiener (329), (330), (332), and Wiechowski (324)), due to the presence of an enzyme, uricase, located chiefly in the liver, and occasionally in the spleen and kidneys of lower animals (Wells (317), Battelli and Stern (25)). By means of perfusion experiments, Ackroyd (6), (8) has shown that the surviving liver of the rabbit quantitatively transforms uric acid into allantoine. Allantoine has been detected also in the blood of lower animals (Hunter (115)); and is the end-product, rather than an intermediate stage, of purine metabolism in these forms, as evidenced by the fact that it undergoes no further oxidation when administered preformed (Ackroyd (6), Taylor and Adolph (292)).

In man uric acid is believed to be the end-product of purine metabolism. Minkowski (232) was unable to detect allantoine in human urine, even after purine administration. More recent investigations (Wiechowski (326), (327)) have shown that small amounts of this substance (10 to 25 mgm. per day) are present in the urine of man, but doubtless have their origin in preformed allantoine of the food (Ackroyd (7)). As might be anticipated from these facts, attempts to demonstrate the presence of uricase in human tissues have been unsuccessful (Battelli

and Stern (25), Miller and Jones (229), Wells and Corper (321), (322), Wiechowski (326)). From the evolutionary point of view, it is of interest to note that anthropoid apes also neither excrete allantoine nor contain the uricolytic enzyme in their tissues (328), (320). Monkeys, on the other hand, are provided with uricase (317), and eliminate allantoine as the chief product of their purine metabolism (116), (117). In this respect monkeys appear to be more closely related generically to the lower animals than to man or the ape. The unique position occupied by man and the ape, as regards purine metabolism, is beautifully

TABLE 2*

The "uricolytic indexes" of mammals

ORDER AND SPECIES	INDEX	ORDER AND SPECIES	INDEX
Marsupialia:		Ungulata:	
Opossum	79	Cow	93
Rodentia:		Horse	88
Rat	96	Sheep	80
Mouse	98	Goat	92
Guinea pig	94	Pig	98
Rabbit	95	Proboscidea:	
Carnivora:		Elephant	72
Raccoon	95	Primates:	
Black bear	94	Monkey	89
Badger	98	Chimpanzee	0
Cat	97	Man	2
Coyote	97		
Dog	98		
Dingo dog	96		
Dalmatian coach dog	32		

* Table taken from Hunter and Ward (120).

illustrated in table 2, taken from Hunter and Ward (120). Hunter and his associates (119), (118), (120) have determined the so-called "uricolytic index" for twenty-two species of animals. This index is the ratio of the output of allantoine nitrogen to the sum of the output of allantoine and uric acid nitrogen. The figures indicate that while the uricolytic indexes for man and the ape are practically zero, these values for other mammals range from 32 to 98.

Although uricolysis in the ordinary meaning of the term has not been possible of demonstration in man, and as pointed out by Fine (64) would seem to be contraindicated by the presence of relatively large

amounts of uric acid in human blood and tissues, the common experience of workers in this field is that administered purines are not quantitatively recovered in the excreta, either as uric acid or as unchanged purines (cf. Kikuchi (144)). This fact is exemplified in a carefully controlled experiment of Taylor and Rose (293). The subject of the experiment, a healthy young adult, was placed upon a diet of exactly 10 grams of nitrogen and 2,000 calories per day. During the first three days, milk, eggs, starch and sugar were the sole foods. For the following three days, a fraction of the milk-egg was substituted by sweetbreads containing the same amount of nitrogen. During the next four days, this substitution was doubled; and during the last four days the subject

TABLE 3*

Thymus feeding and uric acid excretion in man

	PERIOD I 3 DAYS	PERIOD II 3 DAYS	PERIOD III 4 DAYS	PERIOD IV 4 DAYS
Total urinary N	8.90	8.70	9.10	8.80
Urea N + Ammonia N	7.30	7.10	7.10	7.05
Creatinine N	0.58	0.55	0.56	0.47
Total purine N	0.11	0.17	0.26	0.10
Uric acid N	0.09	0.14	0.24	0.07
Rest N	0.91	0.88	1.18	1.18
Nitrogen input	10.0	10.0	10.0	10.0
Purine N input	0	0.17	0.34	0
Average fecal N output	0.5	0.5	0.5	0.5

* Table taken from Taylor and Rose (293).

was again placed upon the diet of the first period. Thus the nitrogen input of the tests was derived as follows:

First period: milk-egg nitrogen, 10 grams.

Second period: milk-egg nitrogen, 7 grams; sweetbread nitrogen, 3 grams.

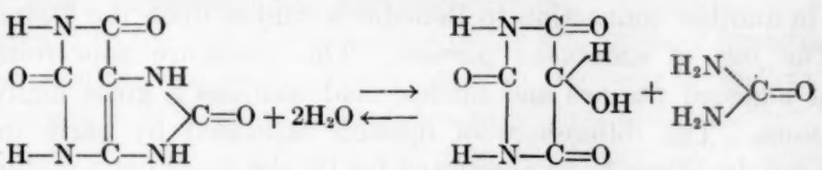
Third period: milk-egg nitrogen, 4 grams; sweetbread nitrogen, 6 grams.

Fourth period: milk-egg nitrogen, 10 grams.

Table 3 presents the results of the experiment in terms of nitrogen daily. Judging by the regularity in output of fecal nitrogen, the digestion and absorption of the sweetbreads was normal and complete. Tests of the feces for purines never showed the presence of more than minute traces. The possibility of bacterial decomposition of purines

with the production of ammonia, as suggested by Thannhauser and Dorfmüller (299), is not excluded, but if such a disintegration occurred to an appreciable extent in our experiment, it is surprising that the urinary urea-ammonia output was not increased. It is clear that the increments in urinary purine nitrogen represent less than half of the known input. The data raise the important question as to whether uric acid may not be oxidized in the human organism without the production of allantoine. Certainly the possibility of such an occurrence has not been excluded.

In regard to uricolysis, the investigations of Ascoli and his associates (18), (19), (38), (123), (125), (254) require brief consideration. These investigators report that incubated liver tissue of animals destroys uric acid with the production of dialuric acid and urea; and in an atmosphere of carbon dioxide reforms uric acid from the disintegration products. The reversible reaction involved is represented as follows:



Dialuric acid

They believe that the active agents responsible for uric acid formation are a thermostable body in the tissues, and a thermolabile enzyme in the blood, both of which are said to be necessary for the synthesis (124). These observations are difficult to correlate with the known facts of purine metabolism. If true, they serve to complicate the situation as regards allantoine, for according to the Italian investigators, this substance, in contrast to dialuric acid, is neither formed from nor transformed into uric acid under the conditions of their experiments. Recently Spiers (281) has denied Ascoli's results *in toto*. It must be admitted, however, that in studies of this sort positive findings are of much greater value than are negative results. The entire question should be reinvestigated.

An interesting racial anomaly of purine metabolism was discovered by Benedict (32) in the case of the Dalmatian dog. In contrast to other dogs, the Dalmatian, upon a purine-free diet, excretes almost as much uric acid per day as does a man upon a similar régime. Benedict has shown that uric acid injected subcutaneously is quantitatively excreted as such in the urine. Wells (319) and Givens (quoted by

Wells) have confirmed Benedict's findings, and the former has made a study of the purine enzymes in the Dalmatian hound. He finds that uricase is present in the liver, indicating that the excretion of uric acid is not due to the absence of the uricolytic ferment. Watanabe (314) also has reported analyses upon two such dogs. He found uric acid in the urine, but in much smaller quantities than observed by other investigators. Possibly his dogs were not of pure Dalmatian breed. Onslow (248) has recently shown that hybrids resulting from the cross of a Dalmatian with another breed of dog excrete uric acid and allantoine in the same proportions as ordinary dogs. According to this author, the greater power of destruction of uric acid is a dominant hereditary character. Benedict's discovery of the metabolic peculiarities of the Dalmatian is not only of scientific interest, but is also of extraordinary practical importance in that it provides an experimental animal whose purine metabolism is similar to that of man. Reference will be made in another connection to Benedict's studies upon the Dalmatian.

c. The fate of exogenous purines. The literature concerning the fate of ingested purines and nucleic acid contains a great many contradictions. The differences of opinion expressed by early investigators are doubtless to be accounted for by the inadequate methods of analysis employed in the separation and identification of the purines, by the use of different species of animals with variable enzyme equipment, and by the failure to appreciate the relationship of allantoine to purine metabolism. Thus Stadthagen (283) and Gumlich (98) observed no increase in the output of uric acid in dogs following the feeding of yeast nucleic acid. The former sought for allantoine in the urine, but failed to detect the slightest trace, while apparently the latter did not consider allantoine formation as a possibility, despite the fact that this substance had been known as an ingredient of urine since the days of Wöhler (335). Later investigations, as will be seen below, have shown that in most mammals uric acid excretion is somewhat increased, and allantoine excretion greatly increased by nucleic acid feeding, but it is not surprising that the small increments in the former should have been overlooked in many experiments on account of the inaccuracies of the analytic methods. Nevertheless, increases in uric acid elimination following the feeding of nucleic acids, or foods containing them, were observed in man by Richter (258), Kühnau (172), Weintraud (315), Umber (306), Hess and Schmoll (106), Weiss (316), Jerome (126), Burian and Schur (51), Loewi (207), Mendel, Underhill

and White (224), and others. Recently Thannhauser and Schaber (302) have reported that following the injection of 1 mgm. doses of adenosine and guanosine in man, recoveries of the purines in the form of uric acid varied in four of the five subjects between 88.8 and 119.9 per cent. The fifth subject, an individual from a gouty family, though he himself had no clinical symptoms of the disease, excreted in two experiments extra uric acid to the extent of only 47.3 and 61.3 per cent of the administered purine. Comparable recoveries of injected nucleosides had previously been obtained in man by Thannhauser and Bommes (296). Less pronounced increases in uric acid following nucleic acid feeding were observed in animals by several investigators, notably Cohn (53) and Mendel and Brown (219), who were also able to demonstrate marked increases in the output of allantoine. Burian, and Schur (51) first emphasized the fact that urinary uric acid is derived partly from body cells and partly from the diet, and named these two fractions "endogenous" and "exogenous" uric acid respectively.

In regard to the fate of free purines in the diet, pioneer investigations yielded results even more chaotic than in the case of nucleic acids. Administration of guanine or xanthine to rabbits and dogs was said to be without effect upon uric acid excretion by Ke:ner (143), Nencki and Sieber (241), Baginski (22), and Krüger and Salomon (167). Even Burian and Schur (51), despite their important observations concerning the effect of exogenous nucleic acids, failed to detect an increase in uric acid output following guanine feeding in man. Hall (100) was inclined to believe that the negative results were due to the relative insolubility of the purines employed, which limited their absorption. That this point was well taken is indicated by the subsequent observations of Schittenhelm and Bendix (270). These authors obtained increases in the output of uric acid following the parenteral administration of guanine in rabbits. Nevertheless, the idea became more or less current that free purines, if catabolized at all, are not transformed to the same end-products as are combined purines (nucleic acids). On the contrary, Minkowski (231) and v. Mach (212) proved that the subcutaneous administration of hypoxanthine in geese causes a marked increase in urinary uric acid. Their experiments are of particular interest in that they constitute the first demonstration of the ability of the animal organism to transform a free oxy-purine into uric acid. In later investigations Minkowski (232) showed that in dogs and men, as well as in birds, hypoxanthine is a precursor of uric acid. Horbaczewski

(111), (112) obtained similar results with hypoxanthine and xanthine. Unfortunately he erred in believing that the leucocytes are the sole source of urinary uric acid, and that the effect of the hypoxanthine or xanthine administration was an indirect one in that purines were supposed by him to occasion a leucocytosis.

Confirmatory evidence that exogenous purines, whether free or combined, are transformed into uric acid (or allantoine) was provided by Krüger and Schmid (171), Mendel, Underhill and White (224), and Mendel and Lyman (222). Mendel, Underhill and White showed that the adenine and guanine of nucleic acid are deaminized and oxidized to uric acid even when the nucleic acid is administered per rectum. Mendel and Lyman found that the oral administration of purines in man led to increases in the output of uric acid amounting to about 60 per cent of the hypoxanthine, 50 per cent of the xanthine, 25 per cent of the guanine, and 34 per cent of the adenine. The adenine experiments of Mendel and Lyman are of particular interest in view of the supposed absence of adenase in the tissues of man. Inspection of their data affords no opportunity of explaining the increases in uric acid output on the basis of a toxicity leading to an exaggerated endogenous nuclear catabolism. The increases in nitrogen elimination following adenine administration are no greater than may be accounted for by the nitrogen present in the purine itself. Nephritic symptoms, accompanied by the formation of 6-amino-2-8-dioxy-purine and its deposition in the kidneys, have been reported in animals following adenine administration (cf. Minkowski (232), Nicolaier (247), Ebstein and Bendix (61)). No abnormal effects were observed in Mendel and Lyman's experiments upon men, but occasionally albuminuria was detected in some of their animal investigations. It may be possible for the organism, in the absence of adenase, to catabolize adenine to uric acid by oxidation in positions 2 and 8 followed by hydrolytic deamination, instead of by direct deamination of the purine with the production of hypoxanthine.

In an interesting paper concerning the fate of free purines in the organism of the monkey, Hunter and Givens (117) have shown that the subcutaneous administration of adenine, guanine, hypoxanthine, or xanthine; as well as of sodium nucleate, leads in each case to slight increases in the output of uric acid and free purines, and to a marked increase in the elimination of allantoine. Recoveries of the injected purines varied from 69 to 105 per cent. The significant data are reproduced below in table 4. The figures in the last column represent

approximations only. In the case of guanine, adenine, and sodium nucleate, account is taken of the fact that uric acid and allantoine contain but four-fifths of the original amino-purine nitrogen; and of the two alternatives offered in the table for the percentage recovery, the lower figures rest upon the assumption that the extra bases are amino-purines, while the higher assume the presence of oxy-purines. The data clearly show that in the monkey free purines of the diet are catabolized, at least in part, just as are combined purines of nucleic acids.

TABLE 4*

Catabolism of exogenous purines in the monkey

PERIOD	SUBSTANCE	PURINE N ADMINISTERED	RECOVERED				
			Milligrams nitrogen				Per cent of theory
			Allantoine	Uric acid	Bases	Total	
		mgm.					
80	Uric acid	36	8	14		22	61
110	Uric acid	49	12	23		35	71
89	Guanine	71	38	2	20	60	99–105
100	Xanthine	56	35	2	17	54	97
97	Adenine	64	15	3	21	39	69–76
85	Hypoxanthine	50	28	3	9	40	80
107	Sodium nucleate	90	38	3	26	69	88–96

* Table taken from Hunter and Givens (117).

The investigations of both Mendel and Lyman and Hunter and Givens serve in the main to emphasize the probability that both endogenous and exogenous purines are catabolized through the same intermediary stages, with the production of identical end-products. The source of the purines, whether in cellular catabolism or in absorption from the alimentary tract, would seem to play no part in determining how oxidation shall occur. However, certain experiments reported in the literature are difficult to interpret on the basis of a single catabolic path for endogenous and exogenous purines. Indeed, Hunter and Givens' experiments with uric acid are of interest in this regard. As will be observed in table 4, the injection of uric acid in the monkey led to a comparatively small increase in the output of allantoine, but to a very decided increase in uric acid excretion. In both of the experiments recorded, about 40 per cent of the uric acid reappeared unchanged in the

urine. From the high uricolytic index of the monkey, and from the behavior of the purines other than uric acid, one would have expected a larger proportion of the latter to have been oxidized to allantoine. The authors suggest that the high output of unaltered uric acid may be related to the fact that in the monkey uricase is confined to one organ, namely, the liver (cf. Wells (317)). They believe that parenterally

TABLE 5*

Thymus feeding and uric acid injection in the Dalmatian dog

TOTAL N	UREA + NH_3 N	URIC ACID N	ALLANTOINE N	INCREASE IN URIC ACID N	INCREASE IN ALLANTOINE N	REMARKS
gms.	*gms.*	*gms.*	*gms.*	*gms.*	*gms.*	
4.37	3.86	0.120	0.050			Normal diet
4.54	4.03	0.125	0.050			
4.82	4.36	0.127	0.050			
6.48	5.87	0.177	0.210			
6.86	6.15	0.197	0.210	0.223	0.480	Same diet + 100 gms. thymus daily = 0.32 gms. purine N
7.30	6.58	0.200	0.210			
5.75	5.27	0.129	0.058			Normal diet
5.54	5.08	0.132	0.058			
5.27	4.84	0.137	0.058			
5.18	4.50	0.290	0.115			Same diet + 500 mgm. of uric acid subcutaneously
5.13	4.47	0.299	0.142			
5.00	4.30	0.305	0.133			
5.02	4.54	0.124	0.066			

* Table taken from Benedict (32).

introduced uric acid may escape contact with the enzyme before elimination has occurred. On the contrary, if this be true it is exceedingly difficult to understand why uric acid formed from injected purine bases does not likewise escape oxidation.

Another observation of interest in this connection is that of Benedict (32) upon the purine metabolism of the Dalmatian coach hound. This author compared the effect of thymus feeding and uric acid injection upon urine composition. As will be seen in table 5 taken from Benedict's article, thymus feeding caused a much larger increase in the output of allantoine than of uric acid. On the purine-free diet the

uric acid nitrogen was more than double that of the allantoine, while after the thymus ingestion the increase in uric acid nitrogen was only about one-half the increase to be found in the allantoine nitrogen. These data, as pointed out by Benedict, may perhaps be interpreted as indicating different paths of catabolism of endogenous and exogenous purines. Furthermore, the subcutaneous administration of uric acid in Benedict's experiment was followed by a marked increase in the allantoine output, despite the fact that the uric acid was quantitatively recovered as such in the urine. Evidently the uric acid administration induced an increased allantoine excretion. On the basis of these results Benedict states that "it seems probable that uric acid and allantoine are interrelated in metabolism in other ways than have been heretofore assumed." Results somewhat analogous to those of Benedict had been obtained previously in a different species of animal by Ackroyd (9). This investigator found a rise in the output of allantoine in the rat following the injection of hypoxanthine, although Rohdé and Jones (260) were unable to detect xanthine oxidase in the total mixed tissues of the rat. Jones (130) remarks that "it is conceivable that hypoxanthine can 'induce' allantoine in the organism of the rat" in a manner analogous to the effect of uric acid upon allantoine elimination in the Dalmatian dog. The situation regarding purine metabolism in the rat is further complicated by the fact that the urine of this animal habitually contains rather large quantities of uric acid (cf. Folin and Morris (86)). To account for the presence of the latter, one must assume its formation by means of reactions which do not involve xanthine oxidase.

Finally must be mentioned the work of Hirokawa (108), who showed that the feeding of thymus tissue to dogs is followed by an excretion of allantoine which is almost quantitative, while the increase in uric acid is very small. If, however, the high purine ration is continued for several weeks, there results a gradual decrease in allantoine, accompanied by a tenfold increase in uric acid.

The work of Benedict, Ackroyd and Hirokawa serves to emphasize the unsatisfactory state of our knowledge of purine metabolism, and to point to the necessity of further investigations before answers will be found to the many questions connection with the catabolic fate of free and combined, endogenous and exogenous purines.

d. The excretion of uric acid and purines. The uric acid arising in the catabolism of endogenous and exogenous purines is in man excreted in the urine chiefly as mono-urates. The total output per day depends

upon the quantity of purines or purine-containing foods ingested in the diet. On a purine-free ration it usually amounts to 0.30 to 0.50 grams *per diem* (cf. Folin (77)), but is subject to fairly wide variations in different individuals. Indeed, in the same individual the endogenous uric acid elimination is not as constant from day to day as was once believed. The quantity and kind of purine-free food play an important part in determining the output. This will be discussed in another connection.

Excretion is less during the night than during the day (259), (174), (52). Determinations of the hourly elimination show that the maximum output is reached in the fore-noon, and is followed by a gradual decline until the very early morning, when an increase to the maximum again occurs (244), (52).

Diseases in which there is an exaggerated nuclear catabolism also usually lead to more or less marked increases in the excretion of endogenous uric acid. Two illustrative conditions of this sort are leucemia (142), (272), and pneumonia (142).

At various times in the past muscular activity has been said to increase the output of endogenous uric acid (cf. Burian 48)). This idea, which had been practically abandoned, has been revived in recent years by Raiziss, Dubin and Ringer (256), and Kikuchi (145). Rakestraw (257) reports slight increases in the uric acid of the blood following exercise. Probably the balance of evidence, however, is against the view that moderate exertion influences purine metabolism. Unquestionably, work of a very strenuous or unaccustomed nature may increase uric acid elimination.

In addition to uric acid, human urine contains small quantities of other purines, the total amount approximating 15 to 45 mgm. per day (Flatow and Reitzenstein (76)). We are indebted to Krüger and Salomon (168), (169) for our knowledge of these purines. From 10,000 liters of urine these investigators prepared 94.49 grams of purine bases having the following composition:

1-7-Dimethyl xanthine	15.310 grams
1-Methyl-xanthine	31.285 grams
7-Methyl-xanthine	22.345 grams
7-Methyl-guanine	3.400 grams
Adenine	3.540 grams
Hypoxanthine	8.500 grams
Xanthine	10.110 grams
	94.490 grams

As will be observed, more than two-thirds of the total bases were methyl-xanthines, which do not occur as ingredients of nucleic acids. Evidently they have their origin in the three methyl-purines of beverages, namely, caffein of coffee, theobromine of cocoa, and theophylline of tea. These substances, which are respectively 1-3-7-trimethyl-xanthine, 3-7-dimethyl-xanthine, and 1-3-dimethyl-xanthine, undergo partial demethylation in the body with the production of mono- and di-methyl-purines (cf. Albanese (11), (12), Bondzynski and Gottlieb (44), (45)). Apparently in man as in the rabbit (166), the methyl group in position 3 is most readily removed, and that in position 7 is most difficult to remove. The methyl group in position 1 is intermediate in stability. Demethylation of caffein, theobromine, the theophylline in position 3, respectively, doubtless accounts for the methyl-purines of the urine. In the dog the conditions are the reverse of those which pertain in man and the rabbit. In this species (165) demethylation is most readily accomplished in position 7, and next in the case of position 1. The methyl group in position 3 is most resistant. Methyl-purines other than the three which occur naturally, and even dioxy-purines other than xanthine, may undergo no change whatever in passage through the organism (94). This fact emphasizes the observation made above, that purine enzymes are remarkably specific in their action.

Numerous attempts have been made in the past to show an increase in uric acid elimination in man and animals following the administration of methyl-purines. Most of these experiments have yielded negative results (232), (50), (170), (273). Taylor (290) observed increases in uric acid following caffein ingestion. Recently, Benedict (32) obtained a distinct rise in uric acid excretion, both in man and the Dalmatian dog, following the administration of caffein. It appears that in the case of this methyl-purine at least, a small part is completely demethylated and oxidized to uric acid, and that the remainder is excreted as the mono- and di-methyl-xanthines. Perhaps the most interesting feature of Benedict's caffein investigation was the tendency to nitrogen retention observed in both the human and dog experiments. Even comparatively small doses led to distinct falls in nitrogen output. Obviously, there is a twofold reason why methyl-purines should be excluded from the diet of gouty individuals.

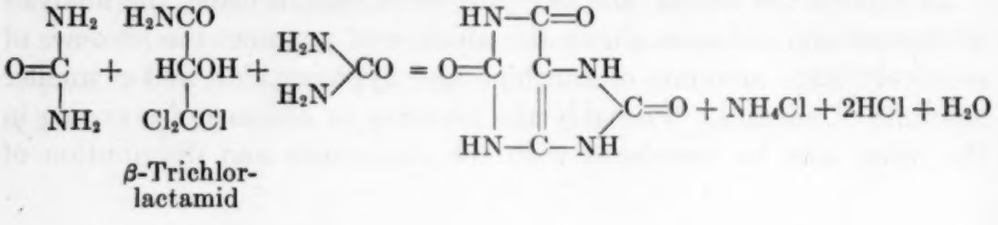
As regards the amino- and oxy-purines of human urine, the analysis of Krüger and Salomon shows the absence of guanine, the presence of relatively large amounts of xanthine and hypoxanthine, and of smaller amounts of adenine. Probably the presence or absence of a purine in the urine may be correlated with the occurrence and distribution of

purine enzymes in the tissues. Guanase is known to be widely distributed in human organs, adenase is supposedly absent, and xanthine oxidase is present in the liver only. These conditions should favor the presence of traces of adenine, hypoxanthine, and xanthine in the urine of man.

Identification of urinary purines other than uric acid also has been made in the monkey, *Cercopithecus callitrichus*, by Hunter (114), and in the pig by Schittenhelm and Bendix (271) and Schittenhelm (269). In these species, as in man, oxy-purines preponderate over amino-purines. Hypoxanthine exceeds xanthine in the urine of the pig, while xanthine exceeds hypoxanthine in the urine of the monkey.

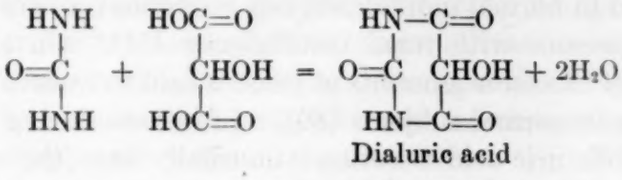
e. Purine catabolism in birds and reptiles. A peculiarity of purine catabolism not observed in mammals is manifested by birds and reptiles. In 1877 von Knieriem (149) showed that the feeding of various amino-acids to hens resulted in increased uric acid excretion. Similar effects were soon found by Schröder (275) to follow the administration of ammonium salts. Meyer (226) showed that urea also can serve as a precursor of uric acid.

Through the investigations of Minkowski (231) and von Mach (212) evidence was presented indicating that urinary uric acid in birds has two distinct origins: *a*, the catabolism of purines as in other animal forms, and *b*, synthesis. According to these investigators, most of the uric acid disappears from the urine of geese, and is replaced by lactic acid and urea, following extirpation of the liver. Small amounts of uric acid continue to be excreted, and represent the true purine catabolism of the bird. On the other hand, in this species, the larger part of the uric acid normally eliminated is an end-product of protein metabolism. The appearance of lactic acid and urea in lieu of uric acid points to the former as precursors of the latter. Urea appears to be formed from amino-acids as in other animals, but instead of being excreted as such, is transformed into uric acid. Complete confirmation of this conception is provided by the investigations of Kowalewski and Salaskin (164), who found that perfusion of the livers of birds with solutions of ammonium lactate or amino-acids results in uric acid synthesis. One is reminded in this connection of Horbaczewski's (110) *in vitro* synthesis of uric acid by heating a mixture of urea and β-trichlorlactamid.

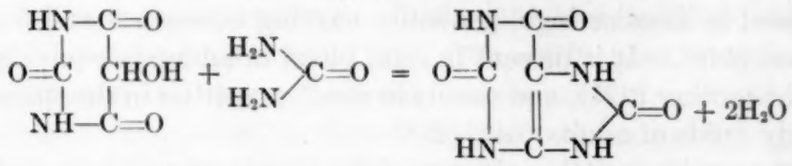


β-Trichlor-lactamid

But according to Wiener (331), the avian liver oxidizes lactic acid to tartronic acid, and condenses the latter with urea as indicated in the equations below:



Dialuric acid



Certainly this method of uric acid formation does not occur extensively in mammals. On the other hand, the possibility of traces of purines arising in this fashion has not been excluded.

6. The uric acid of the blood. Most of the modern investigations of purine metabolism have been directed toward determining the changes in the uric acid content of blood in normal and pathological conditions. Until the past decade our knowledge of blood uric acid was limited practically to the data of Garrod. Between 1848 and 1858, Garrod (90) published several interesting communications in which he described the isolation of uric acid from the blood, first accomplished by him, and pointed out that while normal blood contains only traces of uric acid, the blood of gouty or nephritic individuals shows the presence of several milligrams per 100 cc. He proved that contrary to the current belief at that time, no increase in blood uric acid occurs in rheumatism. Following the work of Garrod, almost no progress was made along this line until 1913, when Folin and Denis (80) devised their colorimetric method for uric acid estimation. This method, and the several improved procedures of Folin (82), (87), (88), (79), Benedict (36), (30), (33), (35), (34), and others (239), (234), applicable to both blood and urine, have been the means of greatly increasing our knowledge of purine metabolism. Folin and Denis (81) found the normal uric acid content of human blood to vary between 0.7 and 3.7 mgm., with an average not far from 2 mgm. per 100 cc. These values have been verified over and over again by other investigators. Thus McLester (217) reports values of 0.5 to 2.9 mgm. per 100 cc. of blood in 15 normal persons on purine-free diets, and Gettler and Baker (91) obtained values of 1 to 3.5 mgm. per 100 cc. in 30 normal individuals. The average normal concentration

is undoubtedly close to 2 mgm. per 100 cc. of blood, but values as low as 0.7 and as high as 3.5 occasionally occur in perfectly healthy individuals. The ingestion of purine-containing foods is usually without effect upon blood uric acid in normal individuals, but occasions more or less marked increases in persons with renal insufficiency (57). On the contrary, the ingestion of excessive amounts of yeast is said to cause a rise in blood uric acid even in normal subjects (89). In infants during the first few days of life, while uric acid excretion is unusually large, the concentration in the blood is likewise high, generally varying between 3 and 5 mgm. per 100 cc. (148). It is present in fetal blood in amounts equivalent to that of the mother (277), and occurs in small quantities in the spinal and other body fluids of adults (64), (238).

In most vertebrates other than man the blood uric acid is exceedingly small. Folin and Denis (81) report values of 0.05 to 0.2 mgm. per 100 cc. in the rabbit, sheep, pig, horse, monkey, ox and cat. Birds, in whom uric acid is the end-product of protein metabolism, are exceptions to the rule of low blood uric acid. The chicken, duck and goose normally have about 4.8 mgm. per 100 cc. (81), (31). In the blood of fishes uric acid also occurs in rather large amounts (58). Teleosts contain about 4 mgm., and elasmobranchs approximately 1 mgm. per 100 cc. The blood of arthropods shows 0.3 to 3.8 mgm. per cent (233).

Of the abnormal variations in the uric acid content of human blood, the most interesting occur in nephritis and gout. Folin and Denis (83) showed that marked increases occur in uremia. At about the same time, Myers and Fine (237) reported values as high as 27 mgm. per 100 cc. of blood in terminal interstitial nephritis. They also pointed out that in 6 cases of gout the uric acid values varied between 3.8 and 5.8 mgm. The marked retention in nephritis leads to a rather even distribution of uric acid in the tissues (64), (318). It is not remarkable that uric acid retention should occur during deficient renal function, but the important fact originally pointed out by Myers, Fine and Lough (239) is that usually uric acid is the *first* nitrogenous waste product to be retained. In incipient interstitial nephritis, before the urea or creatinine show appreciable increases, values of 6 or more milligrams per cent of uric acid may be found. This observation has been verified by many others, notably Baumann, Hansmann, Davis and Stevens (27), who regard the determination of the uric acid concentration in the blood as one of the most delicate indexes of renal function available.

In gout, uncomplicated by nephritis, the uric acid is increased (66), (84), (253), (65), (92), and usually ranges from 4 to 10 mgm. per 100 cc.,

while the values for the urea and creatinine remain practically normal. The blood picture, therefore, is very similar to that in incipient nephritis. Indeed, the similarity is so striking that Fine (65) has raised the question as to whether gout is not a very early manifestation of nephritis, which may or may not further develop. Of interest in this connection are the findings of McClure (215). This author obtained evidence of renal insufficiency in each of six cases of gout, although no clinical symptoms of nephritis were displayed.

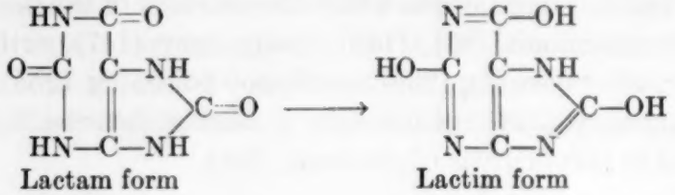
Certain drugs, notably salicylates (68), (55), and 2-phenylquinoline-4 carboxylic acid (atophan) (85), (217), (66), (279) greatly increase the urinary excretion, and lower the blood concentration of uric acid in gout. The increased elimination, however, practically ceases in two days, and more prolonged use of the drug fails to induce further decrease in blood uric acid (66). Probably the drugs act by increasing renal permeability, and perhaps also by accelerating mobilization of uric acid from the tissues (96). In advanced nephritis, atophan has slight or no effect upon uric acid excretion (67). Sodium benzoate has also been said to increase the output, and to lower the concentration in the blood (56), but according to Lewis and Karr (202) its administration may actually lead to decreased elimination.

In addition to gout and nephritis, in which the difficulty is obviously one of renal inefficiency, the uric acid content of blood may be markedly increased in conditions associated with an exaggerated nuclear catabolism. Leucemia (236) is a case in question. Under such circumstances the kidneys may excrete large amounts of uric acid, but apparently are unable to keep pace with its formation. Although in the normal individual there is no threshold concentration for uric acid excretion, such as exists in the case of sugar, nevertheless the kidneys appear to dispose of an oyerload very slowly. Probably this is not due to the relative insolubility of urates, for the blood (291), and usually the urine (105), are able to dissolve at body temperature more uric acid than they are called upon to handle. More likely the lags in excretion, with resulting accumulation in the blood, are associated with the fact, pointed out by Myers and Fine (238), that the kidneys normally are able to concentrate uric acid only about 20 times, while they concentrate urea and creatinine 80 and 100 times respectively.

Other illustrative conditions in which the uric acid of the blood may be increased are pneumonia (83), (146), malignancy (147), arthritis (84), lead and mercury poisoning (236), methanol poisoning (255), and toxemias of pregnancy (333). Curiously, a marked increase has recently been reported in polyneuritis of chickens (304).

As regards the distribution between the plasma and the corpuscles in human blood, uric acid manifests peculiarities which at the present time are impossible of explanation. Of 20 samples of blood examined, Bornstein and Griesbach (46) found 2 with equal distribution, on a volume basis, between the cells and the plasma, 13 in which the uric acid was greater in the cells, and 5 in which it was greater in the plasma. They believe that in shed blood two enzymatic processes occur, one tending to increase, and the other tending to decrease the quantity of uric acid, and that these reactions occur exclusively in the blood cells. In 104 distributional analyses, Theis and Benedict (303) found 51 samples of blood with equal distribution, 8 with greater concentration in the cells, and 45 with larger amounts in the plasma. This relationship was independent of the pathological condition. On adding uric acid to 20 different bloods, these authors found an equal distribution in 30 per cent of the cases, while in 70 per cent the added uric acid did not penetrate the cells at all. They point out that these marked differences in permeability may occur possibly in other cells of the body as well as in the blood corpuscles, and "may tend to throw light on the questions involved in specific uric acid retention in the organism." In analyses of 20 normal bloods, Wu (336) uniformly found about twice as much uric acid in the plasma as in the corpuscles. More recently Plass (250) has reported that in acute retention uric acid accumulates in the plasma first, but later diffuses into the blood cells until the concentration in the latter is practically equal to that in the plasma. Following relief from such a retention, the corpuscles may actually contain more uric acid than the plasma, due to the fact that excretion occurs more speedily than diffusion from the cells into the plasma. It is difficult to harmonize these four observations, and further experiments of a similar nature are needed. It appears not improbable that illuminating results might follow intensive investigations of this sort.

At various times the suggestion has been made that blood may contain more than one form of uric acid. The discovery by Gudzent (97) that uric acid is capable of keto-enol tautomerism, immediately suggested the possibility that different proportions of the so-called *lactam* and *lactim* forms might occur in normal and abnormal bloods. Gudzent believes that only the monosodium salt of the more stable but



Lactam form Lactim form

less soluble lactim form can occur in blood. This idea would appear to be negatived by the finding of amounts of uric acid far in excess of the solubility of the lactim form, which according to Gudzent is only 8.3 mgm. per 100 cc. of serum. One may assume with Bechhold and Ziegler (28) that part of the urate is present in a colloidal condition, but definite proof of this assumption has not been adduced. Of extraordinary interest, therefore, is the discovery of Benedict (31) that ox blood contains a d-ribose-uric acid compound, which he and his associates have succeeded in isolating (54), and which along with the free uric acid, occurs exclusively in the erythrocytes (245). The relatively large amount of this riboside (?) present is indicated by the fact that while fresh ox blood contains only about 0.5 mgm. of free uric acid per 100 cc., 7.5 mgm. may be found after hydrolysis with hydrochloric acid. The uric acid is also liberated by enzymatic action, when the blood is allowed to stand for several days under aseptic conditions.

Continuing the work begun in Benedict's laboratory, Newton and Davis (246) have obtained evidence of the existence of combined uric acid in human, horse, sheep, pig, dog and chicken blood. The quantity in ox blood far exceeds that in the blood of any other animal examined. Human blood appears to be next to ox blood in its combined uric acid content, but only minute traces are detectable in the other species. An interesting distributional difference in the uric acid of ox and chicken bloods was observed by Benedict (31). In contrast to the fact that uric acid in the former is confined to the erythrocytes, in the latter it is almost wholly in the serum.

The ribose-uric acid compound is an exceedingly stable one, and until hydrolyzed does not react with the reagents used in Benedict's colorimetric method. Recently Morris and Macleod (235) have reported the occurrence in certain specimens of human blood of a form of uric acid dimorphism, which they regard as quite distinct from that of Benedict. The evidence which they present is as follows: *a*, The Morris-Macleod method (334) for uric acid estimation gives higher values in some specimens of blood than the procedure of Folin and Wu (87). *b*, From mixed samples of human blood more uric acid can be isolated in crystalline form than is indicated by the Folin-Wu determination. *c*, The addition of excess oxalate to blood samples tends to increase the values obtained by the Folin-Wu method, until they approximate those by the Morris-Macleod procedure. In the writer's judgment the above facts do not prove that blood uric acid is dimorphous in the sense of Morris and Macleod, but are merely *suggestive* of such an occurrence. In any

event, investigations along this line are exceedingly promising. It seems not unlikely that the defect in the metabolism of purines in gout eventually will prove to be associated with either an abnormal distribution, or an unusual form of uric acid in the blood.

7. THE EFFECT OF PURINE-FREE FOODS UPON THE OUTPUT OF ENDOGENOUS URIC ACID. Abundant evidence has been accumulated in recent years to indicate that the original idea of Burian and Schur (51) and Sivén (276), to the effect that the excretion of endogenous purines is constant from day to day in the same individual, is not correct. More than 18 years ago Folin (78) found that the change from a diet of milk and eggs to one of starch and cream might be accompanied by a fall in uric acid elimination of practically 50 per cent, although both diets are purine-free.

Since the work of Folin, numerous investigators, notably Leathes (174), Mendel and Brown (220), Smetánka (278), Taylor and Rose (294), Mendel and Stehle (223), Lewis and Doisy (200), Höst (113) and Rose (262) have corroborated his findings that protein ingestion exerts a marked influence upon urinary uric acid. Smetánka (278), Mendel and Stehle (223), and Umeda (308) also observed an increase in uric acid elimination, as compared with the fasting output, following the ingestion of carbohydrates. Recently Lewis and Corley (199) have reported similar effects from the consumption of honey and glucose syrup in amounts greater than 200 grams, but observed no effects from moderate amounts (100 grams) of glucose, sucrose or lactose. Apparently fats produce the least effect upon purine metabolism of either of the foodstuffs.

The calorific value of the diet is likewise important in determining the uric acid excretion. According to Graham and Poulton (95), diets of protein and fat of insufficient caloric value cause a fall of 30 to 50 per cent in the output of endogenous uric acid. If most of the fat is replaced by carbohydrate no fall is observed. Höst (113) has affirmed that every increase or decrease in the calorific value of the food beyond a certain minimum, is accompanied by a like change in uric acid excretion. Rose (262) also observed distinct increases in uric acid excretion in passing from a low calorific to a high calorific ration, while maintaining the protein intake at a constant level.

No unanimity of opinion exists, however, as to the cause of the increases and decreases in endogenous uric acid elimination induced by changes in the diet. Of the many possible theories offered in explanation of the observed phenomena, the following require brief consideration.

a. Nuclear disintegration occasioned by the work of digestion and food storage. This theory was first suggested by Mareš (214), and was later modified by Smetánka (278). Mareš attributed the increase in output of uric acid following the consumption of purine-free food to nuclear disintegration, chiefly in the alimentary glands, incidental to the physiological work of secretion and digestion. According to this author, uric acid represents the wear and tear of glandular tissues. As evidence for this mechanism, Mareš points not only to the effect of foods, but to the fact that pilocarpine, which is known to increase secretory activity, likewise augments the excretion of uric acid. Lambling and Dubois (173), and Höst (113) also regard digestive work as an important factor in the variations in output of endogenous uric acid, while Mendel and Stehle (223) state that their experiments "offer no obstacle to the assumption that *a portion, at least, of the endogenous uric acid may originate from the activity of the alimentary secretory apparatus.*"

Smetánka, who in the main adheres to the Mareš theory, was forced to modify it. Having observed that the ingestion of honey, a food which requires practically no digestion, causes a marked increase in the output of uric acid, this investigator suggested that in addition to digestive work, the activity involved in glycogenesis may be responsible for a part of the endogenous uric acid. On the other hand, we believe the Mareš-Smetánka theory may be definitely excluded from serious consideration in view of the work of Lewis, Dunn and Doisy (201). These authors showed that glycocoll, alanine, and other amino-acids, which obviously require no digestion, and which in the amounts given could not have formed appreciable quantities of glycogen, increase the hourly output of uric acid as much as do proteins.

b. Stimulated purine synthesis. The well-established fact, considered in some detail above, that the animal organism can synthesize purines in so far as they are required for anabolic purposes, suggests the possibility that these synthetic processes may be stimulated by food ingestion. Indeed Graham and Poulton (95) and Umeda (308) have suggested that part of the endogenous uric acid may arise through synthesis from carbohydrates. They observed that carbohydrate-rich fat-poor diets cause a greater excretion of uric acid than do fat-rich carbohydrate-poor diets, even though the protein content and calorific value of the food are maintained constant. Umeda suggests that uric acid may arise from a condensation of urea with an intermediary product of carbohydrate metabolism, perhaps lactic acid. Graham and Poulton point to the observation of Knoop and Windaus (150), that when glucose is exposed

in vitro to the action of sunlight and the strongly dissociated compound, $Zn(OH)_2 \cdot 4NH_3$, methyl glyoxal and 5-methyl-imidazole are formed. As interesting as these suggestions are, there exists at the present time no experimental evidence *in vivo* which justifies the belief that carbohydrates are transformed into purines in the animal organism.

On the other hand, the possibility of a stimulating effect of proteins upon purine synthesis cannot be excluded so easily. In this connection the work of Ackroyd and Hopkins (10) already referred to, is of special interest. As stated above (p. 560), these authors believe that either arginine or histidine may serve as the substrate for purine formation. A similar conclusion as to the origin of purines in the diamino-acids was arrived at by Harding and Young (104). According to these investigators, the feeding of placenta, which has a high content of arginine, causes a much greater increase in the output of uric acid and allantoine in young dogs than does the ingestion of an equal quantity of muscle protein.

On the contrary, Abderhalden and Einbeck (2), Abderhalden, Einbeck and Schmid (3), and Lewis and Doisy (200) were unable to show any relationship between the diamino-acid content of the diet and the uric acid or allantoine output in the urine. Lewis and Doisy compared the effects of diets high and low in arginine and histidine upon the uric acid output in man. Abderhalden and Einbeck studied the effects of adding histidine to the diet upon the allantoine excretion in the dog. In the later experiment of Abderhalden and his co-workers (3), histidine hydrochloride was given in 10 gram doses to a fasting animal. Neither of the experiments yielded any indication of purine formation from the diamino-acids.

In contrast to the methods of Abderhalden and Lewis and their coworkers, Ackroyd and Hopkins compared the effects of diets *free* from arginine and histidine, with diets containing *adequate amounts* of the diamino-acids. This difference in experimental method is an exceedingly important one. For even if it be admitted that tissue purines have their ultimate origin in arginine and histidine, this fact does not warrant the assumption that the extent of purine synthesis is proportional to the arginine-histidine supply. On the contrary, it seems reasonable to suppose that the synthesis of a tissue component is limited quantitatively to the anabolic needs of the organism for that particular ingredient. As soon as a diet contains sufficient precursors of a given anabolite, synthesis probably occurs at the optimum rate. We believe that this view is entirely in accord with that of Ackroyd and Hopkins. If this

conception is correct, one should not expect an increase in uric acid excretion to follow the feeding of purine precursors, unless the preceding diet were deficient in these precursors. It therefore seems unreasonable to assume a stimulated formation of purines following food ingestion, unless a second assumption is also made, namely, that part of the uric acid in man as in birds is an *end-product of protein catabolism.* We know of no experiments which justify the second proposition.

c. Stimulation of cellular metabolism. In the interesting investigation of Lewis, Dunn and Doisy (201) on the influence of diet upon the hourly elimination of uric acid, the authors suggest that the effect of the ingestion of protein or amino-acids may be due to a general stimulation of cellular metabolism. Each of the four amino-acids, glycocoll, alanine, glutaminic acid, and aspartic acid, as well as the closely related asparagine, caused an appreciable increase in the hourly fasting output of uric acid. The stimulation caused by the dicarboxylic amino-acids was more marked than that produced by glycocoll and alanine. On the other hand, sarcosine, a substituted amino-acid not readily catabolized by the body, and ammonium chloride and urea, were without influence.[4]

For reasons which have been discussed in detail elsewhere (261), we believe that the cellular stimulation theory is the most satisfactory one available at the present time. We (262) have shown that following radical increases in food consumption (particularly as regards the protein portion), the largest alterations in uric acid output occur on the days immediately succeeding the dietary changes. These observations led us to believe that the amino-acids themselves, rather than their intermediary catabolic products, are responsible for the stimulating effect upon cellular activity. On the other hand, the recent findings of Gibson and Doisy (93), that pyruvic acid causes an increase in the hourly excretion of uric acid, and of Lewis and Corley (199) that glycerol exerts a similar effect, indicate that the native foodstuffs and amino-acids are not the only substances which increase uric acid elimination. Moreover, the situation is further complicated by the fact that while pyruvic acid causes an increase, the closely related lactic acid occasions a decrease in uric acid output (93).

[4] Since writing the above, A. A. Christman and H. B. Lewis (Journ. Biol. Chem., 1923, lvii, 379) have reported that the administration of amino-acids in rabbits is followed by *decreases* in allantoine excretion. These observations are in marked contrast to those observed in human subjects, and suggest a possible difference in physiological significance of uric acid in man and of allantoine in lower animals.

It is not likely that in the near future our knowledge will be sufficiently extensive to enable us to accurately picture the details of endogenous purine metabolism, or to fully explain the action of purine-free substances upon uric acid excretion. The problem is a difficult one, and the interpretation of the experimental findings requires unusual caution. But until evidence to the contrary is obtained, the cellular stimulation theory may serve as a helpful working hypothesis.

BIBLIOGRAPHY

(1) Abderhalden, E. Fütterungsversuche mit vollstandig abgebauten Nahrungsstoffen. Zeitschr. physiol. Chem., 1912, lxxvii, 22.

(2) Abderhalden, E. and H. Einbeck. Studien über den Abbau des Histidins im Organismus des Hundes. Ibid., 1909, lxii, 322.

(3) Abderhalden, E., H. Einbeck and J. Schmid. Studien über den Abbau des Histidins im Organismus des Hundes. Ibid., 1910, lxviii, 395.

(4) Abderhalden, E. and T. Kashiwado. Studien über die Kerne der Thymusdrüse und Anaphylaxieversuche mit Kernsubstanzen. (Nucleoproteiden, Nucleinen und Nucleinsäuren.) Ibid., 1912, lxxxi, 285.

(5) Abderhalden, E. and A. Schittenhelm. Der Ab- und Aufbau der Nucleinsäuren im tierischen Organismus. Ibid., 1906, xlvii, 452.

(6) Ackroyd, H. Uric acid metabolism in dogs. Biochem. Journ., 1910–11, v, 217.

(7) Ackroyd, H. On the presence of allantoin in certain foods. Ibid., 1910–11, v, 400.

(8) Ackroyd, H. Uric acid metabolism in rabbits. Ibid., 1910–11, v, 442.

(9) Ackroyd, H. On the purine metabolism of rats. Ibid., 1914, viii, 434.

(10) Ackroyd, H. and F. G. Hopkins. Feeding experiments with deficiences in the amino-acid supply. Arginine and histidine as possible precursors of purines. Ibid., 1916, x, 551.

(11) Albanese, M. Ueber das Verhalten des Coffeins und des Theobromins im Organismus. Arch. exper. Path. u. Pharm., 1895, xxxv, 449.

(12) Albanese, M. Ueber die Bildung von 3-Methylxanthin aus Coffein im thierischen Organismus. Ber. chem. Ges., 1899, xxxii, 2280.

(13) Altmann, R. Ueber Nucleinsäure. Arch. Anat. Physiol., Physiol. Abt., 1889, 524.

(14) Amberg, S. and W. Jones. Über die bei der Spaltung der Nucleine in Betracht kommenden Fermente mit besonderer Beruchsichtigung der Bildung von Hypoxanthin in der Abwesenheit von Adenase. Zeitschr. physiol. Chem., 1911, lxxiii, 407.

(15) Amberg, S. and W. Jones. The action of yeast on yeast nucleic acid. Journ. Biol. Chem., 1912–13, xiii, 441.

(16) Araki, T. Über die Nucleinsäure aus der Schleimhaut des Dünndarms. Zeitschr. physiol. Chem., 1903, xxxviii, 98.

(17) Ascoli, A. Ueber ein neues Spaltungsprodukt des Hefenucleins. Ibid., 1900–01, xxxi, 161.

(18) Ascoli, M. and G. Izar. Quantitative Rückbildung zugesetzter Harnsäure in Leberextrakten nach vorausgegangener Zerstörung. Ibid., 1908, lviii, 529.

(19) Ascoli, M. and G. Izar. Harnsäurebildung in Leberextrakten nach Zusatz von Dialursäure und Harnstoff. Ibid., 1909, lxii, 347.

(20) Baeyer, A. Untersuchungen über die Harnsäuregruppe. Annalen, 1863, cxxvii, 1.

(21) Baeyer, A. Untersuchungen über die Harnsäuregruppe. Ibid., 1863, cxxvii, 199.

(22) Baginski, A. Ueber das Vorkommen von Xanthin, Guanin, und Hypoxanthin. Zeitschr. physiol. Chem., 1883–84, viii, 395.

(23) Bang, I. Die Guanylsäure der Pancreasdrüse und deren Spaltungsprodukte. Ibid., 1898–99, xxvi, 133.

(24) Bang, I. Chemische und physiologische Studien über die Guanylsäure. Ibid., 1901, xxxii, 201.

(25) Battelli, F. and L. Stern. Untersuchungen über die Urikase in den Tiergeweben. Biochem. Zeitschr., 1909, xix, 219.

(26) Bauer, F. Über die Konstitution der Inosinsäure und die Muskelpentose. Beitr. chem. Physiol. Path., 1907, x, 345.

(27) Baumann, L., G. H. Hansmann, A. C. Davis and F. A. Stevens. The uric acid content of the blood compared with the renal dietary test. Arch. Int. Med., 1919, xxiv, 70.

(28) Bechhold, H. and J. Ziegler. Vorstudien über Gicht. III. Biochem. Zeitschr., 1914, lxiv, 471.

(29) Behrend, R. and O. Roosen. Synthese der Harnsäure. Annalen, 1888, ccli, 235.

(30) Benedict, S. R. On the colorimetric determination of uric acid in blood. Journ. Biol. Chem., 1915, xx, 629.

(31) Benedict, S. R. On the uric acid in ox and in chicken blood. Ibid., 1915, xx, 633.

(32) Benedict, S. R. Uric acid in its relations to metabolism. Journ. Lab. Clin. Med., 1916–17, ii, 1.

(33) Benedict, S. R. The determination of uric acid in blood. Journ. Biol. Chem., 1922, li, 187.

(34) Benedict, S. R. The determination of uric acid. Ibid., 1922, liv, 233.

(35) Benedict, S. R. and E. A. Franke. A method for the direct determination of uric acid in urine. Ibid., 1922, lii, 387.

(36) Benedict, S. R. and E. H. Hitchcock. On the colorimetric estimation of uric acid in urine. Ibid., 1915, xx, 619.

(37) Bergmann, T. Opuscula, 1776, iv, 232.

(38) Bezzola, G., G. Izar and L. Preti. Wiederbildung zerstörter Harnsäure in der künstlich durchbuteten Leber. Zeitschr. physiol. Chem., 1909, lxii, 229.

(39) Biberfeld, J. and J. Schmid. Über den Resorptionsweg der Purinkörper. Ibid., 1909, lx, 292.

(40) Biondi, C. Beiträge zur Lehre der fermentativen Prozesse in den Organen. Arch. path. Anat. Physiol., 1896, cxliv, 373.

(41) Bloch, B. Die Umwandlung der Purinkörper im Säugetierorganismus. Biochem. Centralb., 1906, v, 521, 561.

(42) Bloch, B. Die Umwandlung der Purinkörper im Säugetierorganismus. Ibid., 1906, v, 817.

(43) Bloch, B. Die Umwandlung der Purinkörper im Säugetierorganismus. Ibid., 1906, v, 873.

(44) Bondzynski, S. and R. Gottlieb. Ueber Methylxanthin, ein Stoffwechselproduct des Theobromins und Coffeins. Ber. chem. Ges., 1895, xxviii, 1113.

(45) Bondzynski, S. and R. Gottlieb. Ueber die Constitution des nach Coffein und Theobromin im Harne auftretenden Methylxanthins. Arch. exper. Path. u. Pharm., 1896, xxxvii, 385.

(46) Bornstein, A. and W. Griesbach. Über das Verhalten der Harnsäure im überlebenden Menschenblut. Biochem. Zeitschr., 1919–20, ci, 184.

(47) Brunton, L. Organfermente und Organtherapie, eine Prioritätsfrage. Zentralbl. Physiol., 1905–06, xix, 5.

(48) Burian, R. Die Herkunft der endogenen Harnpurine bei Mensch und Säugetier. Zeitschr. physiol. Chem., 1904–05, xliii, 532.

(49) Burian, R. and H. Schur. Ueber Nucleinbildung im Säugethierorganismus. Ibid., 1897, xxiii, 55.

(50) Burian, R. and H. Schur. Ueber die Stellung der Purinkörper im menschlichen Stoffwechsel. Arch. gesammt. Physiol., 1900, lxxx, 241.

(51) Burian, R. and H. Schur. Ueber die Stellung der Purinkörper im menschlichen Stoffwechsel. Ibid., 1901, lxxxvii, 239.

(52) Campbell, J. A. and T. A. Webster. Day and night urine during complete rest, laboratory routine, light muscular work and oxygen administration. Biochem. Journ., 1921, xv, 660.

(53) Cohn, T. Beitrag zur Kenntniss des Stoffwechsels nach Thymusnahrung. Zeitschr. physiol. Chem., 1898, xxv, 507.

(54) Davis, A. R., E. B. Newton and S. R. Benedict. The combined uric acid in beef blood. Journ. Biol. Chem., 1922, liv, 595.

(55) Denis, W. The influence of salicylates on the elimination of uric acid and other waste products from the blood. Journ. Pharm. Exper. Therap., 1915, vii, 255.

(56) Denis, W. The influence of some drugs used in the treatment of gout (and arthritis) on the elimination of uric acid and other waste products from the blood. Ibid., 1915, vii, 601.

(57) Denis, W. The effect of ingested purines on the uric acid content of the blood. Journ. Biol. Chem., 1915, xxiii, 147.

(58) Denis, W. The non-protein organic constituents in the blood of marine fish. Ibid., 1922, liv, 693.

(59) Denis, W. and A. S. Minot. The non-protein nitrogenous constituents of cow's milk. Ibid., 1919, xxxviii, 453.

(60) Denis, W., F. S. Talbot and A. S. Minot. Non-protein nitrogenous constituents of human milk. Ibid., 1919, xxxix, 47.

(61) Ebstein, W. and E. Bendix. Über das Schicksal der in die Blutbahn gebrachten Purinkörper. Arch. path. Anat. Physiol., 1904, clxxviii, 464.

(62) Feulgen, R. Über die Kohlenhydratgruppe in der echten Nucleinsäure. Zeitschr. physiol. Chem., 1914, xcii, 154.

(63) Feulgen, R. Pyrrolreaktion der echten Nucleinsäure. Ibid., 1918, civ, 1.

(64) Fine, M. S. The non-destructibility of uric acid in the human organism. Journ. Biol. Chem., 1915, xxiii, 471.

(65) Fine, M. S. The relation of gout to nephritis as shown by the uric acid of the blood. Journ. Amer. Med. Assoc., 1916, lxvi, 2051.

(66) Fine, M. S. and A. F. Chase. The uric acid concentration of the blood as influenced by atophan and radium emanation. Journ. Pharm. Exper. Therap., 1914–15, vi, 219.

(67) Fine, M. S. and A. F. Chase. The diminished power of the nephritic kidney for eliminating uric acid as exemplified by the use of atophan. Arch. Int. Med., 1915, xvi, 481.

(68) Fine, M. S. and A. F. Chase. The influence of salicylates upon the uric acid concentration of the blood. Journ. Biol. Chem., 1915, xxi, 371.

(69) Fingerling, G. Die Bildung von organischen Phosphorverbindungen aus anorganischen Phosphaten. Biochem. Zeitschr., 1912, xxxviii, 448.

(70) Fischer, E. Verwandlung des Theobromins in methylirte Harnsäuren. Ber. chem. Ges., 1895, xxviii, 2480.

(71) Fischer, E. Ueber die Constitution des Caffeins, Xanthins, Hypoxanthins und verwandter Basen. Ibid., 1897, xxx, 549.

(72) Fischer, E. Neue Synthese der Harnsäure, des Hydroxycaffeins und des Amino-dioxypurins. Ibid., 1897, xxx, 559.

(73) Fischer, E. Synthese des Hypoxanthins, Xanthins, Adenins und Guanins. Ibid., 1897, xxx, 2226.

(74) Fischer, E. Untersuchungen in der Puringruppe (1882–1906). Berlin, 1907.

(75) Fischer, E. and L. Ach. Synthese des Caffeins. Ber. chem. Ges., 1895, xxviii, 3135.

(76) Flatow, R. and A. Reitzenstein. Zur Xanthinbasenbestimmung im Urin. Deutsch. med. Wochenschr., 1897, xxiii, 354.

(77) Folin, O. Approximately complete analyses of thirty "normal" urines. Amer. Journ. Physiol., 1905, xiii, 45.

(78) Folin, O. Laws governing the chemical composition of urine. Ibid., 1905, xiii, 66.

(79) Folin, O. A revision of the method for determining uric acid. Journ. Biol. Chem., 1922, liv, 153.

(80) Folin, O. and W. Denis. A new (colorimetric) method for the determination of uric acid in blood. Ibid., 1912–13, xiii, 469.

(81) Folin, O. and W. Denis. On the uric acid, urea and total non-protein nitrogen in human blood. Ibid., 1913, xiv, 29.

(82) Folin, O. and W. Denis. On the colorimetric determination of uric acid in urine. Ibid., 1913, xiv, 95.

(83) Folin, O. and W. Denis. On the creatinine and creatine content of blood. Ibid., 1914, xvii, 487.

(84) Folin, O. and W. Denis. The diagnostic value of uric acid determinations in blood. Arch. Int. Med., 1915, xvi, 33.

(85) Folin, O. and H. Lyman. On the influence of phenylquinolin carbonic acid (atophan) on the uric acid elimination. Journ. Pharm. Exper. Therap., 1912–13, iv, 539.

(86) Folin, O. and J. L. Morris. The normal protein metabolism of the rat. Journ. Biol. Chem., 1913, xiv, 509.
(87) Folin, O. and H. Wu. A system of blood analysis. Ibid., 1919, xxxviii, 81.
(88) Folin, O. and H. Wu. A revised colorimetric method for determination of uric acid in urine. Ibid., 1919, xxxviii, 459.
(89) Funk, C., W. G. Lyle and D. McCaskey. The nutritive value of yeast, polished rice and white bread as determined by experiments on man. Ibid., 1916, xxvii, 173.
(90) Garrod, A. B. Nature and treatment of gout and rheumatic gout. 2nd ed., London, 1853. Also Med. Chir. Trans., 1848, xxxi, 83; 1854, xxxvii, 49; 1854, xxxvii, 181; 1858, xli, 325. Quoted by V. C. Myers (236).
(91) Gettler, A. O. and W. Baker. Chemical and physical analysis of blood in thirty normal cases. Journ. Biol. Chem., 1916, xxv, 211.
(92) Gettler, A. O. and A. V. St. George. The value of modern blood chemistry to the clinician. Journ. Amer. Med. Assoc., 1918, lxxi, 2033.
(93) Gibson, H. V. and E. A. Doisy. A note on the effect of some organic acids upon the uric acid excretion of man. Journ. Biol. Chem., 1923, lv, 605.
(94) Goldschmidt, S. The metabolism of an isomer of xanthine and some isomers of the methyl-xanthines. Ibid., 1914, xix, 83.
(95) Graham, G. and E. P. Poulton. On the variation in the excretion of endogenous uric acid produced by changes in diet. Quart. Journ. Med., 1913–14, vii, 13.
(96) Griesbach, W. Zur Kritik der Harnsäureausscheidung nach intravenöser Injektion von Harnsäure, mit und ohne Atophan. Biochem. Zeitschr., 1919–20, ci, 172.
(97) Gudzent, F. Physikalische-chemische Untersuchungen über das Verhalten der harnsäuren Salze in Lösungen. Zeitschr. physiol. Chem., 1909, lx, 38.
(98) Gumlich. Ueber die Aufnahme der Nucleine in den thierischen Organismus. Ibid., 1894, xviii, 508.
(99) Haiser, F. and F. Wenzel. Über Karnin und Inosinsäure. Monatsh. Chem., 1908, xxix, 157; 1910, xxxi, 357.
(100) Hall, J. W. A contribution to the knowledge of the purine bodies of human faeces in health and disease. Journ. Path. Bact., 1904, ix, 246.
(101) Hammarsten, O. Zur Kenntniss der Nucleoproteide. Zeitschr. physiol. Chem., 1894, xix, 19.
(102) Hammett, F. S. Concerning the presence of enzymes in muscle tissue which have creatine and creatinine as their substrates. Journ. Biol. Chem., 1922, liii, 323.
(103) Harding, V. J. and R. M. MacLean. A colorimetric method for the estimation of amino-acidα-nitrogen. Ibid., 1916, xxiv, 516.
(104) Harding, V. J. and E. G. Young. Placental feeding and purine metabolism. Ibid., 1919, xl, 227.
(105) Haskins, H. D. The uric acid solvent power of normal urine. Ibid., 1916, xxvi, 205.
(106) Hess, N. and E. Schmoll. Ueber die Beziehungen der Eiweiss und Paranucleinsubstanzen der Nahrung zur Alloxurkörperausscheidung im Harn. Arch. exper. Path. u. Pharm., 1896, xxxvii, 243.

(107) Hesse, A. Zur Bewertung der Schmidt'schen Kernprobe. Zeitschr. exper. Path. Therap., 1909, vii, 91.
(108) Hirokawa, W. Über den Einfluss langdauernder Nucleinsäurefütterung auf den Purinstoffwechsel und die Allantoinausscheidung beim Hunde. Biochem. Zeitschr., 1910, xxvi, 441.
(109) Hoppe-Seyler, F. Ueber die chemische Zusammensetzung der Eiters. Hoppe-Seyler's Med.-chem. Unters., 1871, 486.
(110) Horbaczewski, J. Über eine neue Synthese und die Constitution der Harnsäure. Monatsh. Chem., 1887, viii, 201.
(111) Horbaczewski, J. Untersuchungen über die Entstehung der Harnsäure im Säugethierorganismus. Ibid., 1889, x, 624.
(112) Horbaczewski, J. Beiträge zur Kenntniss der Bildung der Harnsäure und Xanthinbasen, sowie der Entstehung der Leucocytosen im Säugethierorganismus. Ibid., 1891, xii, 221.
(113) Höst, H. F. A study of the physiology of endogenous uric acid. Journ. Biol. Chem., 1919, xxxviii, 17.
(114) Hunter, A. The metabolism of endogenous and exogenous purines in the monkey. Third paper. The purines of monkey urine. Ibid., 1914, xviii, 107.
(115) Hunter, A. The presence of allantoine in mammalian blood. Ibid., 1916–17, xxviii, 369.
(116) Hunter, A. and M. H. Givens. The metabolism of endogenous and exogenous purines in the monkey. Ibid., 1912–13, xiii, 371.
(117) Hunter, A. and M. H. Givens. The metabolism of endogenous and exogenous purines in the monkey. Ibid., 1914, xvii, 37.
(118) Hunter, A. and M. H. Givens. The excretion of purine catabolites in the urine of ungulates. Ibid., 1914, xviii, 403.
(119) Hunter, A., M. H. Givens and C. M. Guion. The excretion of purine catabolites in the urine of marsupials, rodents and carnivora. Ibid., 1914, xviii, 387.
(120) Hunter, A. and F. W. Ward. Comparative studies of purine metabolism in various representative mammals. Trans. Roy. Soc. Canada, 1920, xiii (Sect. 5), 7.
(121) Inouye, K. Über das Vorkommen einer Lävulinsäurebildenen Atomgruppe in Nucleinsäure. Zeitschr. physiol. Chem., 1904, xlii, 117.
(122) Iwanoff, L. Über die fermentative Zersetzung der Thymonucleinsäure durch Schimmelpilze. Ibid., 1903, xxxix, 31.
(123) Izar, G. Beiträge zur Kenntniss der Harnsäurebildung. Ibid., 1910, lxiv, 62.
(124) Izar, G. Beiträge zur Kenntniss der Harnsäurebildung. Ibid., 1910, lxv, 78.
(125) Izar, G. Beiträge zur Kenntniss der Harnsäurezerstörung und -bildung. Ibid., 1911, lxxiii, 317.
(126) Jerome, W. J. S. Further proofs of the origin of uric acid from nuclein-compounds and derivatives. Journ. Physiol., 1899–1900, xxv, 98.
(127) Jones, W. Über das Enzym der Thymusdrüse. Zeitschr. physiol. Chem., 1904, xli, 101.
(128) Jones, W. Concerning nucleases. Journ. Biol. Chem., 1911, ix, 129.

(129) JONES, W. On the physiological agents which are concerned in the nuclein fermentation, with special reference to four independent desamidases. Ibid., 1911, ix, 169.

(130) JONES, W. Nucleic acids, their chemical properties and physiological conduct. 2nd. ed., New York, 1920.

(131) JONES, W. The chemical constitution of adenine nucleotide and of yeast nucleic acid. Amer. Journ. Physiol., 1920, lii, 193.

(132) JONES, W. The action of boiled pancreas extract on yeast nucleic acid. Ibid., 1920, lii, 203.

(133) JONES, W. The thermostable active agent in pig's pancreas. Journ. Biol. Chem., 1922, l, 323.

(134) JONES, W. AND BELT. Unpublished data, quoted by W. JONES (130), p. 96.

(135) JONES, W. AND R. P. KENNEDY. Adenine mononucleotide. Journ. Pharm. Exper. Therap., 1919, xiii, 45.

(136) JONES, W. AND C. L. PARTRIDGE. Über die Guanase. Zeitschr. physiol. Chem., 1904, xlii, 343.

(137) JONES, W. AND M. E. PERKINS. The nucleotides formed by the action of boiled pancreas on yeast nucleic acid. Journ. Biol. Chem., 1923, lv, 557.

(138) JONES, W. AND M. E. PERKINS. The formation of nucleotides from yeast nucleic acid by the action of sodium hydroxide at room temperature. Ibid., 1923, lv, 567.

(139) JONES, W. AND ROWNTREE, L. G. On the guanylic acid of the spleen. Ibid., 1908, iv, 289.

(140) JONES, W. AND M. C. WINTERNITZ. Über die Adenase. Zeitschr. physiol. Chem., 1905, xliv, 1.

(141) KASHIWADO, T. Ein Beitrag zur Kernverdauung und eine Vereinfachung der Schmidt'schen Kernprobe zur Erkennung von Pankreasachylie. Deutsch. Arch. klin. Med., 1911, civ, 584.

(142) KAUFMAN, M. AND L. MOHR. Beiträge zur Alloxurkörperfrage und zur Pathologie der Gicht. Ibid., 1902, lxxiv, 348.

(143) KERNER, G. Ueber das physiologische Verhalten des Guanins. Annalen, 1857, ciii, 249.

(144) KIKUCHI, M. Über das Schicksal des aufgenommenen Purins. Journ. Biochem., 1922, i, 83.

(145) KIKUCHI, M. Über die Bedeutung des Muskels als Quelle des endogenen Purins. Ibid., 1923, ii, 409.

(146) KILLIAN, J. A. Chemical blood changes in pneumonia. Journ. Biol. Chem., 1922, l, xxxvii.

(147) KILLIAN, J. A. AND L. KAST. A study of significant chemical changes in the blood coincident with malignant tumors. Arch. Int. Med., 1921, xxviii, 813.

(148) KINGSBURY, F. B. AND J. P. SEDGWICK. The uric acid content of the blood of new borns. Journ. Biol. Chem., 1917, xxxi, 261.

(149) V. KNIERIEM, W. Ueber das Verhalten der im Säugethierkörper als Vorstufen des Harnstoffs erkannten Verbindungen zum Organismus der Hühner. Zeitschr. Biol., 1877, xiii, 36.

(150) KNOOP, F. AND A. WINDAUS. Über Beziehungen zwichen Kohlenhydraten und stickstoffhaltigen Produkten des Stoffwechsels. Beitr. chem. Physiol. Path., 1905, vi, 392.

(151) KOLLMAN, G. Über Harnsäuresynthese im menschlichen Organismus. Biochem. Zeitschr., 1921, cxxiii, 235.

(152) KOSSEL, A. Ueber das Nuclein der Hefe. Zeitschr. physiol. Chem., 1879, iii. 284.

(153) KOSSEL, A. Ueber das Nuclein der Hefe. Ibid., 1880, iv, 290.

(154) KOSSEL, A. Ueber die Herkunft des Hypoxanthins in den Organismen. Ibid., 1881, v, 152.

(155) KOSSEL, A. Ueber Xanthin und Hypoxanthin. Ibid., 1882, vi, 422.

(156) KOSSEL, A. Ueber Guanin. Ibid., 1883–84, viii, 404.

(157) KOSSEL, A. Ueber eine neue Base aus dem Thierkörper. Ber. chem. Ges., 1885, xviii, 79.

(158) KOSSEL, A. Ueber das Adenin. Ibid., 1885, xviii, 1928.

(159) KOSSEL, A. Weitere Beiträge zur Chemie des Zellkerns. Zeitschr. physiol Chem., 1886, x, 248.

(160) KOSSEL, A. Ueber das Adenin. Ibid., 1888, xii, 241.

(161) KOSSEL, A. Ueber die Nucleinsäue. Arch. Anat. Physiol., Physiol. Abt., 1893, 157.

(162) KOSSEL, A. AND A. NEUMANN. Ueber das Thymin, ein Spaltungsproduct der Nucleinsäure. Ber. chem. Ges., 1893, xxvi, 2753.

(163) KOSSEL, A. AND A. NEUMANN. Darstellung und Spaltungsproducte der Nucleinsäure (Adenylsäure). Ibid., 1894, xxvii, 2215.

(164) KOWALEWSKI, K. AND S. SALASKIN. Ueber die Bildung von Harnsäure in der Leber der Vögel. Zeitchr. physiol. Chem., 1901, xxxiii, 210.

(165) KRÜGER, M. Ueber den Abbau des Caffeins im Organismus des Hundes. Ber. chem. Ges., 1899, xxxii, 2818.

(166) KRÜGER, M. Ueber den Abbau des Caffeins im Organismus des Kaninchens. Ibid., 1899, xxxii, 3336.

(167) KRÜGER, M. AND G. SALOMON. Die Constitution des Heteroxanthins und seine physiologischen Wirkungen. Zeitschr. physiol. Chem., 1895–96, xxi, 169.

(168) KRÜGER, M. AND G. SALOMON. Die Alloxurbasen des Harns. Ibid., 1898, xxiv, 364.

(169) KRÜGER, M. AND G. SALOMON. Die Alloxurbasen des Harns. Ibid., 1898–99, xxvi, 350.

(170) KRÜGER, M. AND J. SCHMID. Der Einfluss des Caffeins und Theobromins auf die Ausscheidung der Purinkörper im Harne. Ibid., 1901, xxxii, 104.

(171) KRÜGER, M. AND J. SCHMID. Die Enstehung der Harnsäure aus freien Purinbasen. Ibid., 1901–02, xxxiv, 549.

(172) KÜHNAU, W. Experimentelle und klinische Untersuchungen über das Verhältniss der Harnsäureausscheidung zu der Leukocytose. Zeitschr. klin. Med., 1895, xxviii, 534.

(173) LAMBLING, E. AND F. DUBOIS. Origin of endogenous purines. Compt. rend. soc. biol., 1914, lxxvi, 614; Chem. Abst., 1917, xi, 622.

(174) LEATHES, J. B. On diurnal and nocturnal variations in the excretion of uric acid. Journ. Physiol., 1906, xxxv, 125.

(175) LEVENE, P. A. Über die Hefenucleinsäure. Biochem. Zeitschr., 1909, xvii, 121.
(176) LEVENE, P. A. The structure of yeast nucleic acid. Journ. Biol. Chem., 1919, xl, 415.
(177) LEVENE, P. A. Crystalline uridinphosphoric acid. Ibid., 1920, xli, 1.
(178) LEVENE, P. A. The structure of yeast nucleic acid. Ibid., 1920, xli, 19.
(179) LEVENE, P. A. Properties of the nucleotides obtained from yeast nucleic acid. Ibid., 1920, xli, 483.
(180) LEVENE, P. A. On the structure of thymus nucleic acid and on its possible bearing on the structure of plant nucleic acid. Ibid., 1921, xlviii, 119.
(181) LEVENE, P. A. AND W. A. JACOBS. Über die Inosinsäure. Ber. chem. Ges., 1908, xli, 2703.
(182) LEVENE, P. A. AND W. A. JACOBS. Über Inosinsäure. Ibid., 1909, xlii, 335.
(183) LEVENE, P. A. AND W. A. JACOBS. Über Inosinsäure. Ibid., 1909, xlii, 1198.
(184) LEVENE, P. A. AND W. A. JACOBS. Über die Pentose in den Nucleinsäure. Ibid., 1909, xlii, 2102.
(185) LEVENE, P. A. AND W. A. JACOBS. Über Guanylsäure. Ibid., 1909, xlii, 2469.
(186) LEVENE, P. A. AND W. A. JACOBS. Über Hefenucleinsäure. Ibid., 1909, xlii, 2474.
(187) LEVENE, P. A. AND W. A. JACOBS. Über Hefenucleinsäure. Ibid., 1909, xlii, 2703.
(188) LEVENE, P. A. AND W. A. JACOBS. Über die Pentose in den Nucleinsäure. Ibid., 1909, xlii, 3247.
(189) LEVENE, P. A. AND W. A. JACOBS. Über die Hefenucleinsäure. Ibid., 1910, xliii, 3150.
(190) LEVENE, P. A. AND W. A. JACOBS. Über die Inosinsäure. Ibid., 1911, xliv, 746.
(191) LEVENE, P. A. AND W. A. JACOBS. Guaninehexoside obtained on hydrolysis of thymus nucleic acid. Journ. Biol. Chem., 1912, xii, 377.
(192) LEVENE, P. A. AND W. A. JACOBS. On guanylic acid. Ibid., 1912, xii, 421.
(193) LEVENE, P. A. AND F. B. LA FORGE. On nucleases. Ibid., 1913, xiii, 507.
(194) LEVENE, P. A. AND J. A. MANDEL. Zur Chemie der Lebernucleoproteide. Über die Guanylsäure. Biochem. Zeitschr., 1908, x, 221.
(195) LEVENE, P. A. AND J. A. MANDEL. Über die Konstitution der Thymonucleinsäure. Ber. chem. Ges., 1908, xli, 1905.
(196) LEVENE, P. A. AND F. MEDIGRECEANU. On nucleases. Journ. Biol. Chem., 1911, ix, 65.
(197) LEVENE, P. A. AND F. MEDIGRECEANU. The action of gastro-intestinal juices on nucleic acids. Ibid., 1911, ix, 375.
(198) LEVENE, P. A. AND F. MEDIGRECEANU. On nucleases. Ibid., 1911, ix, 389.
(199) LEWIS, H. B. AND R. C. CORLEY. The influence of fats and carbohydrates on the endogenous uric acid elimination. Ibid., 1923, lv, 373.
(200) LEWIS, H. B. AND E. A. DOISY. The influence of high protein diets on the endogenous uric acid elimination. Ibid., 1918, xxxvi, 1.
(201) LEWIS, H. B., M. S. DUNN AND E. A. DOISY. Protein and amino-acids as factors in the stimulation of endogenous uric acid metabolism. Ibid., 1918, xxxvi, 9.

(202) Lewis, H. B. and W. G. Karr. The excretion of uric acid in man after ingestion of sodium benzoate. Ibid., 1916, xxv, 13.
(203) Liebig, J. Inosinsäure. Annalen, 1847, lxii, 317.
(204) Liebig, J. and F. Wöhler. Untersuchungen über die Natur der Harnsäure. Ibid., 1838, xxvi, 241.
(205) Lilienfeld, L. Haematologische Untersuchungen ueber das Nuclein. Arch. Anat. Physiol., Physiol. Abt., 1892, 128.
(206) Lilienfeld, L. Ueber Leukocyten und Blutgerinnung. Ibid., 1892, 167.
(207) Loewi, O. Beiträge zur Kenntniss des Nucleinstoffwechsels. Arch. exper. Path. u. Pharm., 1900, xliv, 1.
(208) London, E. S. and A. Schittenhelm. Verdauung und Resorption von Nucleinsäure im Magendarmkanal. Zeits. physiol. Chem., 1910–11, lxx, 10.
(209) London, E. S., A. Schittenhelm and K. Wiener. Verdauung und Resorption von Nucleinsäure im Magendarmkanal. Ibid., 1911, lxxii, 459.
(210) London, E. S., A. Schittenhelm and K. Wiener. Verdauung und Resorption von Nucleinsäure im Magendarmkanal. Ibid., 1912, lxxvii, 86.
(211) Long, E. R. On the presence of adenase in the human body. Journ. Biol. Chem., 1913, xv, 449.
(212) v. Mach, W. Ueber die Bildung der Harnsäure aus dem Hypoxanthin. Arch. exper. Path. u. Pharm., 1888, xxiv, 389.
(213) Marcet. An essay on the chemical history and medical treatment of calcul disorders. London, 1817.
(214) Mareš, F. Der physiologische Protoplasmastoffwechsel und die Purinbildung. Arch. gesammt. Physiol., 1910, cxxxiv, 59.
(215) McClure, C. W. The renal function in gout. Arch. Int. Med., 1917, xx, 641.
(216) McCollum, E. V. Nuclein synthesis in the animal body. Amer. Journ. Physiol., 1909, xxv, 120.
(217) McLester, J. S. Studies on uric acid of blood and urine, with special reference to the influence of atophan. Arch. Int. Med., 1913, xii, 739.
(218) Medicus, L. Zur Constitution der Harnsäuregruppe. Annalen, 1875, clxxv, 230.
(219) Mendel, L. B. and E. W. Brown. Observations on the nitrogenous metabolism of the cat, especially on the excretion of uric acid and allantoin. Amer. Journ. Physiol., 1900, iii, 261.
(220) Mendel, L. B. and E. W. Brown. The rate of elimination of uric acid in man. Journ. Amer. Med. Assoc., 1907, xlix, 896.
(221) Mendel, L. B. and C. S. Leavenworth. Changes in the purine-, pentose-, and cholesterol-content of the developing egg. Amer. Journ. Physiol., 1908, xxi, 77.
(222) Mendel, L. B. and J. F. Lyman. The metabolism of some purine compounds in the rabbit, dog, pig and man. Journ. Biol. Chem., 1910, viii, 115.
(223) Mendel, L. B. and R. L. Stehle. The rôle of the digestive glands in the excretion of endogenous uric acid. Ibid., 1915, xxii, 215.
(224) Mendel, L. B., F. P. Underhill and B. White. A physiological study of nucleic acid. Amer. Journ. Physiol., 1903, viii, 377.

(225) MENDEL, L. B. AND B. WHITE. On the intermediary metabolism of the purine-bodies: The production of allantoin in the animal body. Ibid., 1904, xii, 85.

(226) MEYER, H. Beiträge zur Kenntnis des Stoffwechsels im Organismus der Hühner. Dissertation, Königsberg, 1887.

(227) MIESCHER, F. Ueber die chemische Zusammensetzung der Eiterzellen. Hoppe-Seyler's Med.-chem. Unters., 1871, 441.

(228) MIESCHER, F. Die Spermatozoen einiger Wirbelthiere. Verhandl. d. naturforsch. Ges. in Basel, 1874, vi, 138.

(229) MILLER, J. R. AND W. JONES. Über die Fermente des Nucleinstoffwechsels bei der Gicht. Zeitschr. physiol. Chem., 1909, lxi, 395.

(230) MILROY, T. H. Ueber die Eiweiss-Verbindungen der Nucleinsäure und Thyminsäure und ihre Beziehung zu den Nucleinen und Paranucleinen. Ibid., 1896–97, xxii, 307.

(231) MINKOWSKI, O. Ueber den Einfluss der Leberextirpation auf den Stoffwechsel. Arch. exper. Path. u. Pharm., 1886, xxi, 41.

(232) MINKOWSKI, O. Untersuchungen zur Physiologie und Pathologie der Harnsäure bei Säugethieren. Ibid., 1898, xli, 375.

(233) MORGULIS, S. A study of the non-protein constituents in blood of some marine invertebrates. Journ. Biol. Chem., 1922, l, lii.

(234) MORRIS, J. L. AND A. G. MACLEOD. Colorimetric determination of uric acid. Ibid., 1922, l, 55.

(235) MORRIS, J. L. AND A. G. MACLEOD. Studies on the uric acid of human blood. Ibid., 1922, l, 65.

(236) MYERS, V. C. Practical chemical analysis of blood. St. Louis, 1921.

(237) MYERS, V. C. AND M. S. FINE. The non-protein nitrogenous compounds of the blood in nephritis, with special reference to creatinine and uric acid. Journ. Biol. Chem., 1915, xx, 391.

(238) MYERS, V. C. AND M. S. FINE. Comparative distribution of urea, creatinine, uric acid and sugar in the blood and spinal fluid. Ibid., 1919, xxxvii, 239.

(239) MYERS, V. C., M. S. FINE AND W. G. LOUGH. The significance of the uric acid, urea and creatinine of the blood in nephritis. Arch. Int. Med., 1916, xvii, 570.

(240) NAKAYAMA, M. Über das Erepsin. Zeitschr. physiol. Chem., 1904, xli, 348.

(241) NENCKI, M. AND N. SIEBER. Ueber eine neue Methode die physiologische Oxidation zu messen und über den Einfluss der Gifte und Krankheiten auf dieselbe. Arch. gesammt. Physiol., 1883, xxxi, 319.

(242) NEUBERG, C. AND B. BRAHN. Über Inosinsäure. Ber. chem. Ges., 1908, xli, 3376.

(243) NEUMANN, A. Verfahren zur Darstellung der Nucleinsäure α und β und der Nucleothyminsäure. Arch. Anat. Physiol., Physiol. Abt., Suppl. Bd., 1899, 552.

(244) NEUWIRTH, I. The hourly elimination of certain urinary constituents during brief fasts. Journ. Biol. Chem., 1917, xxix, 477.

(245) NEWTON, E. B. AND A. R. DAVIS. The distribution of the combined uric acid in the corpuscles of beef blood. Ibid., 1922, liv, 601.

(246) Newton, E. B. and A. R. Davis. Combined uric acid in human, horse, sheep, pig, dog and chicken blood. Ibid., 1922, liv, 603.
(247) Nicolaier, A. Über die Umwandlung des Adenins im thierischen Organismus. Zeitschr. klin. Med., 1902, xlv, 359.
(248) Onslow, H. The relation between uric acid and allantoin excretion in hybrids of the Dalmatian hound. Biochem. Journ., 1923, xvii, 334
(249) Osborne, T. B. and L. B. Mendel. Beobachtungen über Wachstum. bei Fütterungsversuchen mit isolierten Nahrungssubstanzen. Zeitschr. physiol. Chem., 1912, lxxx, 307.
(250) Plass, E. D. Variations in the distribution of the non-protein nitrogenous constituents of whole blood and plasma during acute retention and elimination. Journ. Biol. Chem., 1923, lvi, 17.
(251) Plimmer, R. H. A. and F. H. Scott. The transformations in the phosphorus compounds in the hen's egg during development. Journ. Physiol., 1909, xxxviii, 247.
(252) Popoff, P. M. Ueber die Einwirkung von eiweissverdauenden Fermenten auf die Nucleinstoffe. Zeitschr. physiol. Chem., 1894, xviii, 533.
(253) Pratt, J. H. Studies on the uric acid in the blood in gout. Amer. Journ. Med. Sci., 1916, cli, 92.
(254) Preti, L. Beiträge zur Kenntnis der Harnsäurebildung. Zeitschr. physiol. Chem., 1909, lxii, 354.
(255) Rabinovitch, I. M. Biochemical studies in a fatal case of methyl alcohol poisoning. Arch. Int. Med., 1922, xxix, 821.
(256) Raiziss, G. W., H. Dubin and A. I. Ringer. Studies in endogenous uric acid metabolism. Journ. Biol. Chem., 1914, xix, 473.
(257) Rakestraw, N. W. The effect of muscular exercise upon certain common blood constituents. Ibid., 1921, xlvii, 565.
(258) Richter, P. F. Ueber Harnsäureausscheidung und Leukocytose. Zeitschr. klin. Med., 1895, xxvii, 290.
(259) Rockwood, E. W. The elimination of endogenous uric acid. Amer. Journ. Physiol., 1904, xii, 38.
(260) Rohdé, A. and W. Jones. The purine ferments of the rat. Journ. Biol. Chem., 1909–10, vii, 237.
(261) Rose, W. C. The influence of food ingestion upon endogenous purine metabolism. I. Ibid., 1921, xlviii, 563.
(262) Rose, W. C. The influence of food ingestion upon endogenous purine metabolism. II. Ibid., 1921, xlviii, 575.
(263) Rose, W. C. and G. J. Cox. Unpublished data.
(264) Rose, W. C. and G. T. Lewis. Unpublished data.
(265) Sachs, F. Ueber die Nuclease. Zeitschr. physiol. Chem., 1905, xlvi, 337.
(266) Salkowski, E. Bildung von Allantoin aus Harnsäure im Thierkörper. Ber. chem. Ges., 1876, ix, 719.
(267) Scheele. Opuscula, 1776, ii, 73.
(268) Scherer. Ueber einen im thierischen Organismus vorkommenden, dem Xanthicoxyd verwandten Körper. Annalen, 1850, lxxiii, 328.
(269) Schittenhelm, A. Über den Nucleinstoffwechsel des Schweines. Zeitschr. physiol. Chem., 1910, lxvi, 53.
(270) Schittenhelm, A. and E. Bendix. Ueber die Umwandlung des Guanins im Organismus des Kaninchens. Ibid., 1904–05, xliii, 365.

(271) Schittenhelm, A. and E. Bendix. Vergleichende Untersuchungen über die Purinkörper des Urins beim Schwein, Rind und Pferd. Ibid., 1906, xlviii, 140.

(272) Schmid, J. Ein Beitrag zum Stoffwechsel bei der chronischen Leukämie. Deutsch. Arch. klin. Med., 1903, lxxvii, 505.

(273) Schmid, J. Der Abbau methylierter Xanthine. Zeitschr. physiol. Chem., 1910, lxvii, 155.

(274) Schmidt, A. Die klinische Bedeutung der Ausscheidung von Fleischresten mit dem Stuhlgang. Deutsch. med. Wochenschr., 1899, xxv, 811.

(275) Schröder, W. Ueber die Verwandlung des Ammoniaks in Harnsäure im Organismus des Huhns. Zeitschr. physiol. Chem., 1878–79, ii, 228.

(276) Sivén, V. O. Zur Kenntniss der Harnsäurebildung im menschlichen Organismus unter physiologischen Verhältnissen. Skand. Arch. Physiol., 1901, xi, 123.

(277) Slemons, J. M. and L. J. Bogert. The uric acid content of maternal and fetal blood. Journ. Biol. Chem., 1917, xxxii, 63.

(278) Smetánka, F. Zur Herkunft der Harnsäure beim Menschen. Arch. gesammt. Physiol., 1911, cxxxviii, 217.

(279) Smith, C. A. and P. B. Hawk. Action of atophan and novatophan in gout and iritis. Arch. Int. Med., 1915, xv, 181.

(280) Socin, C. A. In welcher Form wird das Eisen resorbirt? Zeitschr. physiol. Chem., 1891, xv, 93.

(281) Spiers, H. M. The supposed synthesis of uric acid from its decomposition products by tissue extracts. Biochem. Journ., 1915, ix, 337.

(282) Spitzer, W. Die Ueberführung von Nucleinbasen in Harnsäure durch die sauerstoffübertragende Wirkung von Gewebsauszügen. Arch. gesammt. Physiol., 1899, lxxvi, 192.

(283) Stadthagen, M. Ueber das Vorkommen der Harnsäure in verschiedenen thierischen Organen, ihr Verhalten bei Leukämie und die Frage ihrer Entstehung aus den Stickstoffbasen. Arch. path. Anat. Physiol., 1887, cix, 390.

(284) Steudel, H. Über die Oxydation der Nucleinsäure. Zeitschr. physiol. Chem., 1906–07, l, 538.

(285) Steudel, H. Über die Guanylsäure aus der Pankreasdrüse. Ibid., 1907, liii, 539.

(286) Steudel, H. Über die Kohlenhydratgruppe in der Nucleinsäure. Ibid., 1908, lv, 407.

(287) Steudel, H. Über die Kohlenhydratgruppe in der Nucleinsäure. II. Ibid., 1908, lvi, 212.

(288) Strauch, F. W. Die Grundlage der Ad. Schmidt'schen Kernprobe. Deutsch. Arch. klin. Med., 1910–11, ci, 128.

(289) Strecker, A. Ueber das Sarkin. Annalen, 1858, cviii, 129.

(290) Taylor, A. E. The influence of various diets upon the elimination of the urinary nitrogen, urea, uric acid, and the purine bases. Amer. Journ. Med. Sci., 1899, cxviii, 141.

(291) Taylor, A. E. On the solubility of uric acid in blood serum. Journ. Biol. Chem., 1906, i, 177.

(292) Taylor, A. E. and W. H. Adolph. On uricolysis. Ibid., 1914, xviii, 521.

(293) Taylor, A. E. and W. C. Rose. On uricolysis in the human subject. Ibid., 1913, xiv, 419.

(294) Taylor, A. E. and W. C. Rose. The influence of protein intake upon the formation of uric acid. Ibid., 1914, xviii, 519.

(295) Thannhauser, S. J. Verdauung der Hefenucleinsäure durch menschlichen Duodenalsaft. Isolierung der Triphosphonucleinsäure. Zeitschr. physiol. Chem., 1914, xci, 329.

(296) Thannhauser, S. J. and A. Bommes. Stoffwechselversuche mit Adenosin und Guanosin. Ibid., 1914, xci, 336.

(297) Thannhauser, S. J. and G. Czoniczer. Kennen wir Erkrankungen des Menschen die durch eine Störung des intermediären Purinstoffwechsels verursacht wird? Deutsch. Arch. klin. Med., 1921, cxxxv, 224.

(298) Thannhauser, S. J. and G. Dorfmüller. Über den Aufbau des Hefenucleinsäuremolekules und seine gleichartig Aufspaltung durch milde, ammoniakalische und fermentative Hydrolyse. Zeitschr. physiol. Chem., 1917, c, 121.

(299) Thannhauser, S. J. and G. Dorfmüller. Über die Aufspaltung des Purinringes durch Bakterien der menschlichen Darmflora. Ibid., 1918, cii, 148.

(300) Thannhauser, S. J. and G. Dorfmüller. Die Aufspaltung von Nucleotiden durch wäserige Pikrinsäurelösung in der Hitze. Isolierung der krystallisierten Cytidinphosphorsäure. Ibid., 1919, civ, 65.

(301) Thannhauser, S. J. and P. Sachs. Experimentelle Studien über den Nucleinstoffwechsel. Die Desamidierung der Triphosphonucleinsäure. Ibid., 1920–21, cxii, 187.

(302) Thannhauser, S. J. and H. Schaber. Zur Frage der intermediären Urikolyse beim Menschen. Ibid., 1921, cxv, 170.

(303) Theis, R. C. and S. R. Benedict. Distribution of uric acid in blood. Journ. Lab. Clin. Med., 1921, vi, 680.

(304) Thompson, T. J. and I. L. Carr. The relation of certain blood constituents to a deficient diet. Biochem. Journ., 1923, xvii, 373.

(305) Tichomiroff, A. Chemische Studien über die Entwicklung der Insecteneier. Zeitschr. physiol. Chem., 1885, ix, 518.

(306) Umber, F. Ueber den Einfluss nucleinhaltiger Nahrung auf die Harnsäurebildung. Zeitschr. klin. Med., 1896, xxix, 174.

(307) Umber, F. Ueber die fermentative Spaltung der Nucleoproteide im Stoffwechsel. Ibid., 1901, xliii, 282.

(308) Umeda, N. The influence of fat and carbohydrate on the excretion of endogenous purines in the urine of the dog and man. Biochem. Journ., 1915, ix, 421.

(309) Underhill, F. P. and I. S. Kleiner. The influence of hydrazine upon intermediary metabolism in the dog. Journ. Biol. Chem., 1908, iv, 165.

(310) Unger, B. Das Guanin und seine Verbindungen. Annalen, 1846, lix, 58.

(311) Virchow, R. Ueber Concretionen im Schweinfleisch, welche warscheinlich aus Guanin bestehen. Arch. path. Anat. Physiol., 1866, xxxv, 358.

(312) Virchow, R. Die Guanin-Gicht der Schweine. Ibid., 1866, xxxvi, 147.

(313) Voegtlin, C. and C. P. Sherwin. Adenine and guanine in cow's milk. Journ. Biol. Chem., 1918, xxxiii, 145.

(314) WATANABE, C. K. A comparative study of the rate of excretion of the nitrogenous waste products to their blood concentrations in experimental uranium nephritis. Journ. Urol., 1917, i, 485.

(315) WEINTRAUD, W. Ueber den Einfluss des Nucleins der Nahrung auf die Harnsäurebildung. Berl. klin. Wochenschr., 1895, xxxii, 405.

(316) WEISS, J. Weitere Beiträge zur Erforschung der Bedingungen der Harnsäurebildung. Zeitschr. physiol. Chem., 1899, xxvii, 216.

(317) WELLS, H. G. The purine metabolism of the monkey. Journ. Biol. Chem., 1909–10, vii, 171.

(318) WELLS, H. G. The accumulation of uric acid in the tissues during suppression of the urine. Ibid., 1916, xxvi, 319.

(319) WELLS, H. G. The purine metabolism of the Dalmatian coach hound. Ibid., 1918, xxxv, 221.

(320) WELLS, H. G. AND G. T. CALDWELL. The purine enzymes of the orangutan (*Simia satyrus*) and chimpanzee (*Anthropopithecus troglodytes*). Ibid., 1914, xviii, 157.

(321) WELLS, H. G. AND H. J. CORPER. Observations on uricolysis, with particular reference to the pathogenesis of "uric acid infarcts" in the kidney of the new-born. Ibid., 1909, vi, 321.

(322) WELLS, H. G. AND H. J. CORPER. The purines and purine metabolism of the human fetus and placenta. Ibid., 1909, vi, 469.

(323) V. WESTENRIJK, N. Die Kernprobe von Prof. Ad. Schmidt. Zeitschr. exper. Path. Therap., 1910, viii, 353.

(324) WIECHOWSKI, W. Die Produkte der fermentativen Harnsäurezersetzung durch tierische Organe. Beitr. chem. Physiol. Path., 1907, ix, 295.

(325) WIECHOWSKI, W. Die Bedeutung des Allantoins im Harnsäurestoffwechsel. Ibid., 1908, xi, 109.

(326) WIECHOWSKI, W. Über die Zersetzlichkeit der Harnsäure im menschlichen Organismus. Arch. exper. Path. u. Pharm., 1909, lx, 185.

(327) WIECHOWSKI, W. Das Schicksal intermediärer Harnsäure beim Menschen und der Allantoingehalt des menschlichen Harns: nebst Bemerkungen über Nachweis und Zersetzlichkeit des Allantoins. Biochem. Zeitschr., 1910, xxv, 431.

(328) WIECHOWSKI, W. Ein Beitrag zur Kenntnis des Purinstoffwechsels der Affen. Prag. med. Wochenschr., 1912, xxxvii, 275.

(329) WIENER, H. Ueber Zersetzung und Bildung der Harnsäure im Thierkörper. Arch. exper. Path. u. Pharm., 1899, xlii, 375.

(330) WIENER, H. Die Harnsäure. Ergebn. Physiol., 1902, i, 555.

(331) WIENER, H. Über synthetische Bildung der Harnsäure im Tierkörper. Beitr. chem. Physiol. Path., 1902, ii, 42.

(332) WIENER, H. Die Harnsäure in ihrer Bedeutung für die Pathologie. Ergebn. Physiol., 1903, ii, 377.

(333) WILLIAMS, J. L. Increased amount of uric acid in the blood in the toxemias of pregnancy. Journ. Amer. Med. Assoc., 1921, lxxvi, 1297.

(334) WINTERNITZ, M. C. AND W. JONES. Über den Nucleinstoffwechsel mit besonderer Berücksichtigung der Nucleinfermente in den menschlichen Organen. Zeitschr. physiol. Chem., 1909, lx, 180.

(335) WÖHLER, F. Allantoin im Kalberharn. Annalen, 1849, lxx, 229.

(336) WU, H. Separate analyses of the corpuscles and the plasma. Journ. Biol. Chem., 1922, li, 21.

THE MODERN THEORY OF GENETICS AND THE PROBLEM OF EMBRYONIC DEVELOPMENT

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The several attempts that have been made in the past to "explain" the development of the individual from the egg by postulating physiological units, pangenes, determinants and other kinds of representative particles have, not without reason, called forth a good deal of criticism as to the value of such a procedure, especially in the biological sciences where the evidence for an assumption of this kind was largely wanting.

The disrepute into which these philosophical speculations fell has carried over, and there has remained, in certain quarters at least, a prejudice against the modern theory of heredity, based on Mendel's laws, that also postulates units or elements in the germinal materials. A little discrimination might have shown that because certain speculations concerning developmental units had little evidence in their support, and were objectionable on more than one score, it did not follow that the egg and sperm might not contain discrete elements, chemical compounds, through whose perpetuity heredity is possible and through whose effects the process of embryonic development might be influenced. It was scarcely rational to reject all theories assuming the germ-cell to contain a number of definite substances that are the agents through which heredity takes place, because a few philosophers had postulated fictitious units. The value of any hypothesis relating to heredity and to development will depend of course on the kind of evidence that can be brought to its support.

Without attempting to review the history of the theories of representative particles that have played a rôle in modern biological speculations, I shall first point out the kind of evidence on which our interpretation of embryonic development rests and then give the evidence from genetics that leads to the conclusion that heredity is due to discrete particles in the germinal material. If I can succeed in removing some of the unnecessary prejudices that seem still to persist on the part of those not familiar at first hand with the evidence, I shall have

accomplished the purpose of this article. While it is not possible to bring forward here more than a fraction of the evidence on which the modern theory of heredity rests, it should be possible, even in a limited space, to indicate the kind of evidence that supports this theory.

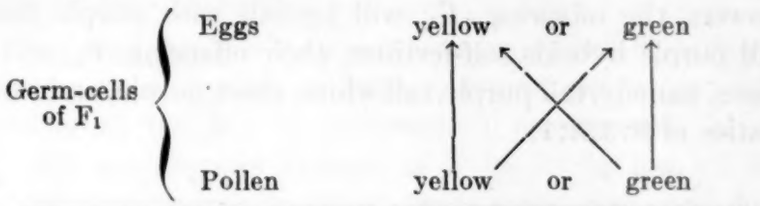
The assumptions that underlie our modern ideas as to the relation between genetics and development are the following: *a*. The orderliness of the developmental process is a fact of observation. The eggs of an individual develop into the same kinds of individuals, if the environment is constant. *b*. Since vastly different kinds of animals (species) develop in the same environment, in the sea, or in a lake, for instance, their differences must depend on differences in the eggs themselves. *c*. If an alteration takes place in the composition of an egg, the end product of its development is expected to be different from what it was before, and if this alteration should be present again in the eggs of the next generation, the same effects are expected. An alteration of this kind is called a mutation. These generalizations rest on actual experience and, as will probably be conceded by everyone familiar with the evidence, they mean no more than that the developmental process is regarded as a causal phenomenon, or in other words, it appears to be an orderly sequence of recurrent events.

When, however, we come to enquire more specifically into the details on which these generalities are based, we find that we have not penetrated very far into the nature of the changes that are postulated. Nevertheless it can be shown, I think, that there are sufficient grounds for holding such views.

The evidence for the statement that at times a change may take place in an egg (or sperm cell) of such a sort that it persists in the eggs (and sperm cells) of succeeding generations, is derived from new types that suddenly appear and reappear in the descendants of such mutant types. Before bringing forward this evidence, there is a further relation that should be considered. It is this. Between the egg and the end product of its development—the individual—we are familiar with a long series of events as a consequence of which a specific form emerges. While there is an extensive literature describing these stages, it is generally admitted that we know very little as to the nature of the changes that take place. Our idea that they are causal events rests largely, as I have said, on their orderliness, which is the same for countless individual cases. Are we justified, then, in postulating that a change in the germ-cell has occurred whenever a new type appears that is, in a sense, permanent? Since every individual starts as an

egg, there seems to be no escape from this interpretation, which is moreover in accord with a vast body of information showing that the egg of an individual is a cell that has been derived by successive generations from a similar cell (the egg) of the preceding generation.

The evidence that something in the germ-material that is correlated with typical characters perpetuates itself, is derived from Mendel's law of segregation. This may be illustrated as follows: If a yellow pea, (P_1,) is crossed to a green pea, (P_1,) the peas produced by the cross (F_1) are yellow. If these are self-fertilized, their offspring, F_2, consists of yellow and green peas in the ratio of three yellows to one green. When these, F_2, in turn are tested, it is found that the green peas breed true to green, and that there are two kinds of yellows in the ratio of two to one. The former, if selfed, produce again yellow and green peas in the same ratio of 3:1, while the latter, if selfed, breed true to yellow. Mendel pointed out that these numerical results can be explained by a very simple assumption. The original yellow pea, P_1,. carries something in its germ-cells that makes it yellow, and the green pea, something in its germ-cells that makes it green. When the two are brought together in the first generation hybrid, F_1, the result is not a blend of the two, but the yellow dominates. (This, however, is of no importance, for in many other cases the hybrid is intermediate.) If when the germ-cells of the hybrid come to maturity, the two somethings (that made one type yellow and the other type green) separate (segregate), then half of the germ cells will be yellow-producing and half will be green-producing.



F_2 1. yellow yellow ; 2. yellow green : 1. green green

3. yellow : 1. green

Chance fertilization of any egg by any pollen grain will give three kinds of combinations, namely, *1*, yellow yellow; *2*, yellow green; *3*, green green. Of these three combinations the first will be pure for yellow, the second (which are twice as numerous) will be mixed yellow and green in composition, but yellow in expression, since, as shown in the first generation, when both are present the yellow dominates; the third will be pure for green. These classes are in the ratio of 1:2:1.

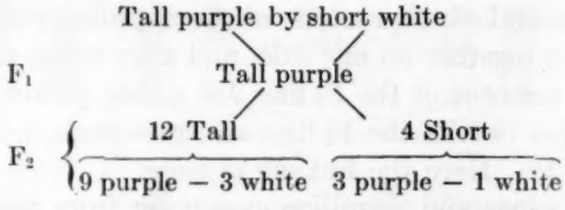
Mendel's theory rests, therefore, on the assumption that if in the germ-cells of the hybrid the two parental contributions—the yellow-producing and the green-producing contributions—separate (segregate) so that half of the germ-cells are of one kind and half of the other, and if the chances are equal that any pollen grain may fertilize any egg, the observed numerical results in the second generation are accounted for.

A simple experiment of this kind reveals nothing as to the nature of the germinal material of the two parents. For instance, it would not be necessary to assume that there is an element for yellow and one for green, although obviously this would be the commonplace way of stating the relation; for when, as here, the assumption of a difference between the two kinds of peas suffices for the theory, there is nothing to demonstrate that there must be representative units in the germ material that stand for yellow or green. The explanation would be exactly the same if it were stated that all that went into the hybrid (from the yellow parent) separated from all that went into the hybrid (from the green parent). But this interpretation will not cover all the results found by Mendel, as is made clear by those cases where two or more character-differences are found in the same cross. This evidence is furnished by Mendel's second law, or law of independent assortment of the genes. It may also be illustrated by an example.

Tall and dwarf peas form a Mendelian pair of characters, i.e., when crossed they give in F_2 a 3 to 1 ratio, etc. Purple flower color and white flower color are another pair of such characters. If a pea that is tall and has purple flowers is crossed to a pea that is short and has white flowers, the offspring, F_1, will be tall with purple flowers. If these tall purple hybrids self-fertilize, their offspring, F_2, will fall into four classes, namely, tall purple, tall white, short purple, and short white, in the ratios of 9:3:3:1.

Pollen \ Eggs	T P	t P	T p	t p
T P	T P T P tall purple	t P T P tall purple	T p T P tall purple	t p T P tall purple
t P	T P t P tall purple	t P t P short purple	T p t P tall purple	t p t P short purple
T p	T P T p tall purple	t P T p tall purple	T p T p tall white	t p T p tall white
t p	T P t p tall purple	t P t p short purple	T p t p tall white	t p t p short white

It is obvious that the result can not be explained, as in the case where a single pair of characters only is involved, by assuming that the whole germ material of the original tall purple parent separates in the germ-track of the hybrid from the germ material of the short white parent; but it is equally obvious that, as Mendel pointed out, the results can be explained if we treat each pair of characters that entered the cross as though it behaved independently of the other pair of characters. For example, if the F_2 offspring are separated into talls and shorts, they will be as 3 to 1. Amongst the talls again, there should be purples and whites in the ratio of 3 purple and 1 white, and among the shorts there should also be found three purples to 1 white. Since there are three times as many talls as shorts, the expectation for all the offspring is 9 tall purple, 3 tall white, 3 short purple, and 1 short white. The following diagram represents these relations.



Mendel also found that when three pairs of characters enter a cross, each pair behaves independently of the other two pairs, or, stated more accurately, the numerical results obtained, when three pairs of characters are present, can be accounted for, if each pair of characters segregates independently of the segregation of the other two pairs.

Mendel's second law has been shown to be limited in its application. A new phenomenon, known as linkage, places serious restrictions on the generality of the law of independent assortment. Mendel did not meet with any cases of linkage, or if he did he has left no statements in regard to them. In recent years many such cases have been found, including a few in garden peas. However, the free assortment shown by two pairs of characters is proof that the germ-materials that went in and segregated in the hybrid are not wholes, but must be treated as separable into parts. How far this material may be subdivided can only be determined at present by the genetic evidence.

The evidence from free assortment carries us only a short distance in the analysis of the divisibility of the germ-materials. It has led, however, in recent years to a very important conclusion, namely, that the number of independent factor-pairs corresponds with the

number of the chromosome pairs. In other words, the evidence from Mendel's second law has shown that the divisibility of the hereditary material extends as far as the chromosomes. But to-day we go far beyond this conclusion, and we are able to do so because there has been discovered another phenomenon, crossing-over, that enables us to work within the linkage groups.

Linkage and crossing-over. The evidence from genetics shows that in many cases when two characters are present in the same cross they do not freely assort, but the characters that went in from the one or from the other parent tend to remain together in subsequent generations. If they always remain together they are said to be completely linked; when they separate occasionally, they may be said to be closely linked; when they separate more freely, they are loosely linked. A few examples will bring out these relations more clearly.

When the sex-linked characters of Drosophila, yellow wings and white eyes, go in together on one side, and gray wings and red eyes on the other, 98.5 per cent of the F_2 flies are either yellow white or gray red. Only 1.5 per cent of the F_2 flies are cross-overs, i.e., yellow red or gray white (fig. 1). Here the linkage is close.

When yellow wings and vermilion eyes enter from one side and gray wings and red eyes from the other side, 69 per cent of the F_2 offspring are the classes that went into the cross together, namely, yellow vermilion and gray red, and 31 per cent of the F_2 flies are cross-overs, namely, yellow red and gray vermilion. Here the linkage is not so close.

When yellow wings and bar eyes enter from one side and gray wings and round eyes from the other side, 44 per cent of the F_2 offspring are the classes that went in together, namely, yellow round and gray bar. This is a case of loose linkage. Similar examples could be given from each of the other three linkage groups of Drosophila.

These facts do not reveal one of the most fundamental discoveries relating to linkage, which is apparent only when representatives of a sufficiently large number of linked characters enter the cross at the same time to enable us to detect the nature of the process that is taking place. When such an experiment is made, the results show for a given linkage series, such, for example, as the sex-linked series of *Drosphila*, that no crossing-over at all occurs in 43 per cent of the eggs, and that crossing-over occurs once in about 43.5 per cent of the eggs.[1]

[1] Crossing over occurs twice in 13 per cent of the eggs and three times in 0.5 per cent.

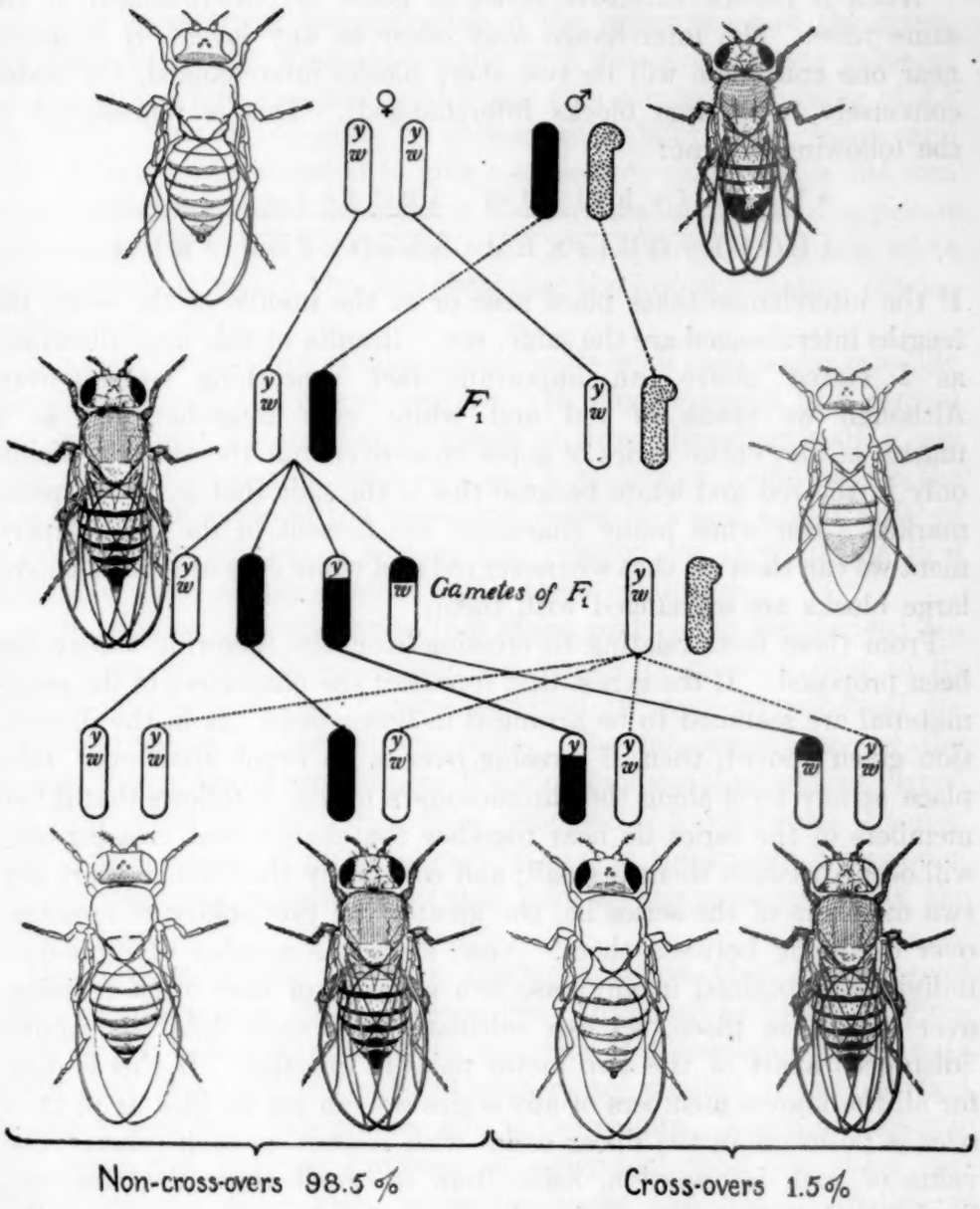


Fig. 1. Cross of a female Drosophila melanogaster that has white eyes and yellow wings to a wild type male with red eyes and gray wings. The F_1 daughters are like their mother and the F_1 sons are like the father. In the lower line of the diagram the four kinds of F_2 flies are shown. The first two are like their grand parents and represent non-cross-over classes. The other two are the cross-over flies.

When it occurs, extensive series of genes are interchanged at the same time. The interchange may occur at any level. If it occurs near one end there will be two short blocks interchanged, (or stated conversely, two large blocks interchanged). This is represented in the following scheme:

a b c d e f g h i j k l m	A B C d e f g h i j k l m
A B C D E F G H I J K L M	a b c D E F G H I J K L M

If the interchange takes place near or at the middle of the series the lengths interchanged are the same, etc. Results of this kind illustrate, as I stated above, an important fact concerning crossing-over. Although we speak of red and white eyes interchanging, as a matter of fact entire series of genes cross-over, but the event is visible only in the red and white because this is the pair that is, so to speak, marked. But when many characters are present in the same experiment we can then see that whenever red and white eyes are interchanged large blocks are exchanged with them.

From these facts relating to crossing-over, the following theory has been proposed. If the genes, that represent the characters in the germ-material are assumed to be arranged in linear order (as in the illustration given above), then, if crossing over is an event that may take place at any level along the chromosome's length, it follows that if two members of the series lie near together the chance that crossing-over will occur between them is small; and conversely the further apart any two members of the series lie, the greater the probability of crossing-over occurring between them. Now, since the number of cross-over individuals obtained in any case is a measure of how often crossing-over has taken place, we can calculate from such data the *relative* "distance" apart of the two factor pairs in question. If this is done for all the known members of any series we can get an idea as to their *relative* positions in the linear series with respect to each other. The value of such information, aside from its great theoretical interest, lies in the fact that these assigned numerical values allow us to predict how a new member of any series will behave with respect to all the others of that series, provided its numerical relation to two members of the known series is first determined. This is done by testing its crossing-over with any two members of the series, preferably two that are not too "distant." The ability to predict new combinations is one of the ear-marks of a scientific hypothesis, and in this respect the theory of the linear order of the genes fulfils the requirements.

Instead of trying to justify the theory on these grounds, there is a logical or geometrical demonstration of the linear order of the genes. For example, yellow wings and white eyes give a cross-over value of 1.2 per cent, white eyes and bifid wings give a cross-over value of 3.5 per cent. Now if the order is yellow white bifid, then yellow and bifid, if tested, are expected to give a cross-over value that is the sum of the other two; but if the order is white yellow bifid, then the yellow bifid cross-over value is expected to be the difference between white bifid and yellow bifid. The data show that the former situation obtains in this case.

This relation for three or more points holds in all cases so far carefully investigated with the proper controls. It may be stated in a general way, as follows: The cross-over values of three characters a, b, c, are such that the cross-over value of a and b is always found to be the sum or the difference of the cross-over values of a and c and that of b and c. This relation is a geometric relation for three points in a line. No other simple relation fulfils these conditions.

It is necessary to emphasize that these conclusions are derived entirely from genetic data, because the theory of crossing over in its further development has become involved in more hypothetical inferences relating to the chromosomes. The full significance of the genetic theory would remain even if the chromosomal inferences turned out to be quite different from those proposed. Nevertheless, I think, that it is a matter of great interest to find in the known behavior of the chromosomes certain stages that fulfil all the requirements of the genetic theory.

Double crossing-over. There is genetic evidence that crossing-over may take place twice in the same series. Inasmuch as double crossing-over, as it is called, introduces the necessity of a correction in the data when only two pairs of linked characters are studied, it is important to make clear how this process affects the results. Again an example will serve our purpose: Suppose an individual with the three sex-linked factors, white eyes, miniature wings and forked bristles, is crossed to an individual with the normal allelomorphs of these characters, red eyes, long wings and straight bristles. In the hybrid female the two X-chromosomes bearing the genes for these characters may be represented as follows:

white	miniature	forked
red	long	straight

Now if crossing-over should take place at two levels between white and forked, the relation of the three pairs of genes involved would then be represented as follows:

white	long	forked
red	miniature	straight

If the cross-over value of white-red and forked-straight were alone being studied, the combinations of these characters that come out after such a double crossing-over would be the same as those that went in, namely, white-forked and red-straight as shown in the second diagram above. Consequently, these individuals would be classified as non-cross-overs and two crossings-over would be missing. This would give relatively too low a crossing-over value for this combination in comparison with cases where no double crossing-over occurs, and the percentage values would be imperfect to the extent to which double crossing-over had occurred.

If, however, in such a cross a third character is taken into account—one which previous experiments had shown to be between the extremes—then double crossing-over will be detected when it occurs, for the double crossing-over will give flies that are white long forked and red miniature straight (see diagram).

Experience has shown that when the cross-over value of two factor-pairs is small, no double crossing-over occurs, and that the chance of its occurring increases as the cross-over values become larger. This relation may be stated in a simple way; namely, that when crossing-over takes place at one level the region on each side is protected from a second cross-over in the same egg.

The further away any level lies, the greater is the likelihood of double crossing-over taking place, at least up to a certain distance. It is true, of course, on chance alone, the greater the length of the series the greater the expectation of a second crossing-over occurring, but the observed relations show that chance alone will not account for the facts. The only explanation so far formed involves a consideration of the chromosome behavior and will be described below.

If the chance of a second crossing-over steadily increases as the distance from the first crossing-over increases, a level might be reached which is no longer affected by the first crossing-over. This relation has also been found, by Weinstein, to hold. The evidence for this is important for any consideration dealing with the mechanism of crossing-over; for, whatever hypothesis of crossing-over is suggested, it

must fulfil the requirement of these discovered relations. There is, in fact, a mechanical factor which will account for this relation. If the interchange takes place at the time of conjugation as a result of the twisting or looping of the pair of chromosomes involved, the semi-rigidity of the thread imposes such conditions on the situation.

It will have been observed that cross-over values have been often spoken of as though they could be converted into distance apart of the genes. If understood properly, the use of the more graphic term distance should not lead to confusion, but, since it has been misunderstood even by geneticists, a word of caution may not be superfluous. An imaginary situation will serve to bring out the most essential point. If in one stock, crossing-over should be more frequent between two factor-pairs than between the same factor pairs in another stock the "distance" would appear to be greater in the former. Obviously, under such circumstances distance could not have the precise meaning that it appears to have when cross-over values are converted into distance. Only when the cross-over values are constant could we compare two independently obtained results. Now, as a matter of fact, cross-over values are remarkably constant under known conditions. On the other hand there is abundant evidence to show that crossing-over may be influenced both by external and by internal factors. These can be studied by experimental methods and the corrections necessary to convert one set of observations into another can be obtained. Therefore, in making out tables or "maps" of the linkage groups, it is expedient to exclude those cases where unusual environmental or genetic conditions have been found to be present. The numerical values assigned to the series of linked genes in our maps represent what may be called normal values, i.e., those most frequently met with when known disturbing factors are eliminated.

Distance as a genetic term is, after all, not the most interesting fact brought out by this work in crossing-over, even although the practical aspects of assigning values to the position of the genes in the series are manifest. It is the linear order of the genes and their retention of this order through all the vicissitudes of changes in crossing-over that is the most significant feature of the results. A striking case in point is that studied by Sturtevant. He found that there is a region at the right end of the chromosome extending for a distance of 56 units that is affected by a genetic factor in such a way that crossing-over is cut down almost to zero, but the little crossing-over that remains shows that the order of the genes is not changed.

A recent "map" of the four linkage groups of Drosophila melanogaster is shown in figure 2. Only the more familiar or more used genes are named, the other cross lines indicate the known location of still other genes. In addition to these there are as many more characters known, but they are not so accurately placed as are those here shown. In making these maps the linear order of the series is determined from material as homogeneous as possible, i.e., from pair cultures under favorable environmental conditions and where no known internal disturbing factors are present. The linear order once determined in this way, the cross-over values assigned to the loci are the average results of a great amount of data, often obtained at various times and from various sources, excluding all those cases known to be exceptional. Values obtained in this way give the best approximations to those most likely to be met with.

There is also some evidence to show that crossing-over in certain regions of the linkage group is more likely to occur than in other regions. This disturbs the relative values of the distances, but since this is a constant variant, it does not call for a correction in the maps. For the further working out of the problems of crossing-over, however, the regional difference may be highly important.

The chromosome mechanism. The evidence that the chromosomes are intimately concerned with the inheritance of Mendelian characters has steadily grown more convincing. The fact that the behavior of the chromosomes at the reduction period furnishes a mechanism that parallels the postulates of Mendel's two laws in all respects, would in itself attract the immediate attention of any biologist who was not content to have these laws stand without regard to processes taking place in the eggs and sperm cells. Even were there no other evidence, I tlfink that biologists would have welcomed this application of Mendelian principles to cytology, but there is at present other, direct evidence pointing to the chromosomes as the bearers of the Mendelian genes. The correspondence of the linkage groups in Drosophila melanogaster is important in this connection, for if the chromosomes carry the genes, and if they are, as they appear to be, self-perpetuating bodies in the cell, such a phenomenon as that of linkage is expected. Linkage, in this sense, means only that the chromosome remains a constant element from one generation to the next. What was not to be anticipated on such a view, however, was the genetic discovery that this constancy of the chromosomes holds only as far as other chromosomes are concerned. It does not hold for members of the same pair. Be-

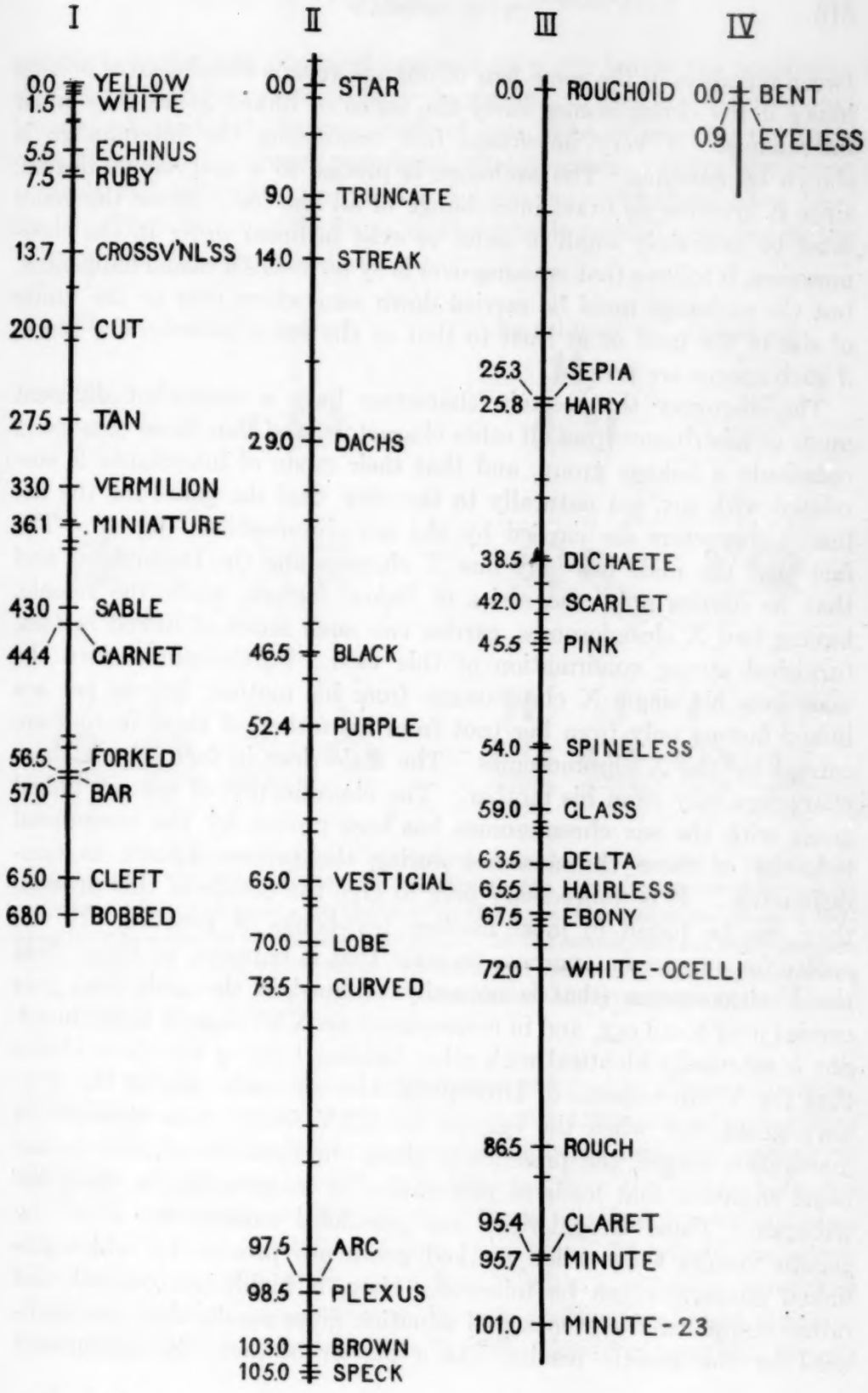


Fig. 2. The four series of linked genes of Drosophila melanogaster. The first, I, is the sex-linked series.

tween members of the same pair of linkage groups crossing-over occurs, hence if the chromosomes carry the series of linked genes they must interchange. A very important fact concerning the interchange is shown by genetics. The exchange is precise to a marvellous degree, since it involves an exact interchange of all like loci. Since the genes must be extremely small in order to exist in linear order in the chromosomes, it follows that crossing-over is by no means a casual happening, but the exchange must be carried down somewhere near to the limits of size of the gene or at least to that of the space between the genes, if such spaces are present.

The discovery that certain characters have a somewhat different mode of inheritance from all other characters, and that these characters constitute a linkage group, and that their mode of inheritance is correlated with sex, led naturally to the view that the genes for the sex linked characters are carried by the sex chromosomes (fig. 3). The fact that the male has only one X chromosome (in Drosophila) and that he carries only one series of linked factors, while the female, having two X chromosomes, carries two such series of linked factors, furnished strong confirmation of this view. Furthermore, since the male gets his single X chromosome from his mother, he can get sex linked factors only from her (not from his father) if these factors are carried by the X chromosomes. The male does in fact inherit these characters only from his mother. The identification of the sex linked genes with the sex chromosomes has been proven by the exceptional behavior of these chromosomes during the process known as nondisjunction. It is unnecessary here to give the details of this process, they can be found in most modern text-books of genetics. It will suffice for our present purpose to state that it happens, at times, that the Y chromosome (that is normally confined in the male line) gets carried over to an egg, and in consequence an XXY female is produced. She is externally identical with other females, bearing out the evidence that the Y chromosome of Drosophila does not carry any of the ordinary genes, but when the eggs of an XXY female pass through the maturation stages, the presence of three chromosomes creates an unusual situation that leads at maturation to irregularities in their distribution. These irregularities are paralleled exactly by what the genetic results show when marked genes are present by which sex-linked characters can be followed. Here, a highly exceptional and rather complicated chromosomal situation gives results that are paralleled by the genetic results. As a matter of fact, the exceptional

genetic behavior was observed first and its study led to the conclusion that three sex chromosomes must be present in the non-disjunctional female. A study of the germ-cells of such females revealed the presence there of the three postulated chromosomes. There are many controls and tests that are possible for this case and these have been made. They leave no doubt as to the genetic result being due to the behavior of the chromosomes.

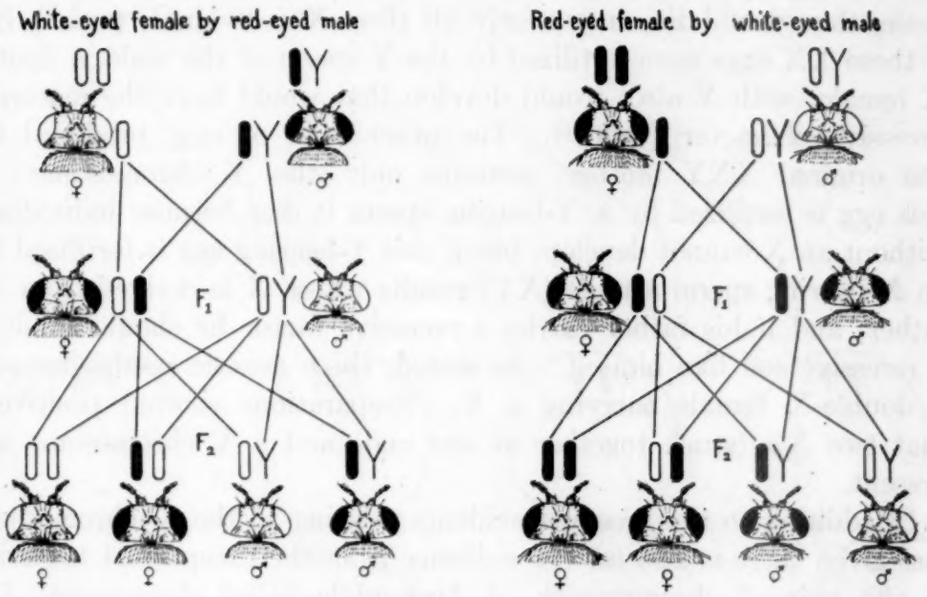


Fig. 3. The inheritance of the sex-linked character, white eyes of Drosophila melanogaster. In the part of the diagram to the left a white-eyed female is mated to a red-eyed male. In the part to the right, the reciprocal cross, a red-eyed female is mated to a white-eyed male. In the center, the sex chromosomes are represented. The white (open) chromosome stands for white eyes and the black chromosome for red eyes. The *Y* stands for the chromosome of that name; it is confined to the male line.

In the process of sex-linked inheritance, referred to above, a mother, if she shows a recessive sex-linked character transmits it to her sons who show it, but if the father carries the sex-linked recessive, neither his daughters nor his sons show it, as is expected from the known behavior of the X chromosomes (see fig. 3). These relations are called criss-cross inheritance. Recently, a case has arisen in which the above relations are exactly reversed—a female with a recessive sex-linked character (yellow) mated to a male with another sex-linked recessive character, produced daughters like herself (instead of sons like herself)

and sons like their father (that carried another recessive). It was apparent from the genetic evidence that the reversal of the ordinary result would be understandable if the two yellow-bearing X chromosomes of such a female were stuck together. If at the same time such a female carried also a Y chromosome then after maturation half of her eggs would retain the double X chromosomes (yellow bearing), and half would retain the Y chromosome (fig. 4). Now if the former kind of eggs should be fertilized by the X-bearing chromosome of the male, they would die, since nearly all three-X individuals perish; but if these XX eggs were fertilized by the Y sperm of the male, a double X female (with Y also) would develop that would have the maternal recessive character (yellow). The other kind of egg, produced by the original XXY mother, contains only the Y chromosome. If this egg is fertilized by a Y-bearing sperm it dies because individuals without an X cannot develop; but if this Y-bearing egg is fertilized by an X-bearing sperm a male (XY) results whose X is derived from his father; and if his father carries a recessive factor he should produce a recessive son like himself. As stated, these genetic results demand a double-X female carrying a Y. Preparations showed positively that two X's (stuck together at one end) and a Y chromosome are present.

In addition to the breeding evidence relating to the X chromosome just given there is also further evidence from the exceptional behavior of the pair of chromosomes of Drosophila called chromosome IV. As shown in figure 5a, there is present in the middle of the metaphase chromosome group of the normal fly a small pair of chromosomes that was supposed for a long time to be the bearers of the smallest group of linked genes. There is now both genetic and cytological evidence fulfilling this prediction. A summary of the results will serve to show not only the kind of changes in heredity brought about by the addition or by the loss of a member to this pair of chromosomes but also the general effect of such changes on development.

There are several ways in which the addition of a chromosome may take place—generally it is supposed by primary non-disjunction either in the male or female. The addition of a third chromosome in Drosophila (fig. 5b) brings about slight though recognizable departures from the wild type. The eyes of such a triplo-IV fly are smaller, the body color is darker, the wings are narrower. These changes while definite, are so slight that they would ordinarily pass unnoticed. If such a triplo-IV fly is bred to a fly that is normal in type and carries

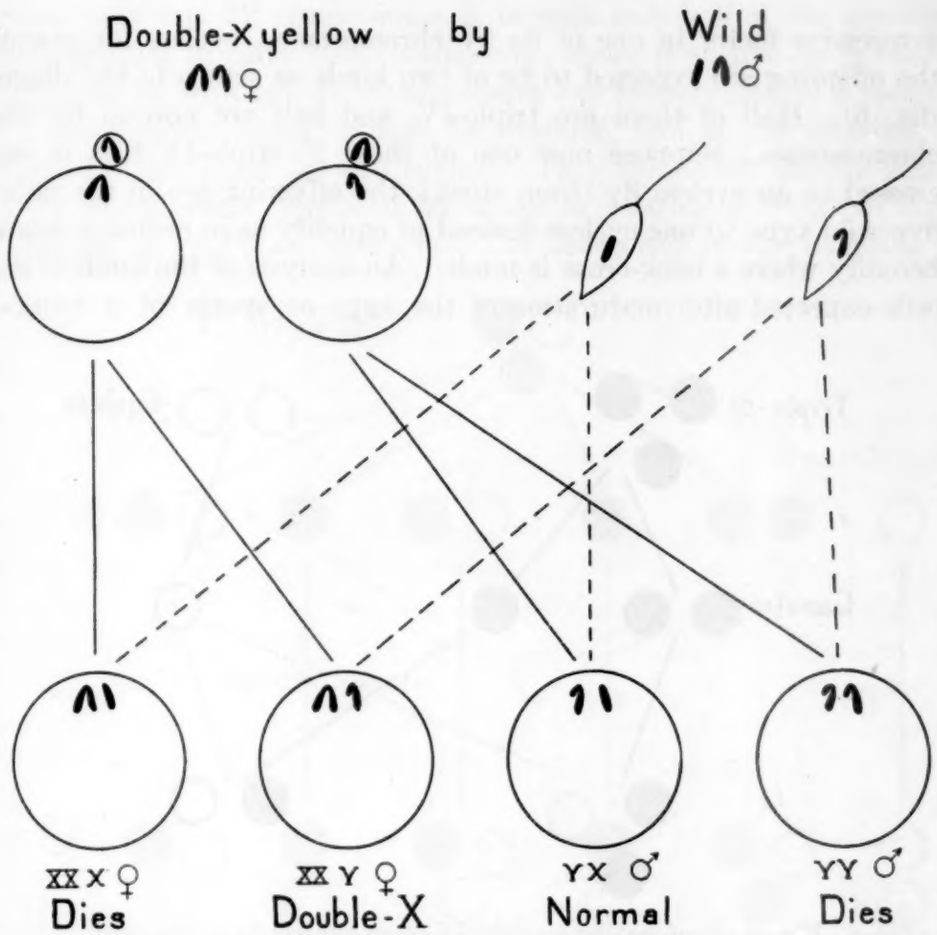


Fig. 4. Diagram to illustrate the two kinds of eggs produced by a double-X, yellow female, and the results of fertilizing them by sperm from a wild type male. The double yellow X-chromosome is represented by the bent loop, and its mate the Y-chromosome by a stippled hook. Four kinds of zygotes are produced (shown in the lower line). Two of these die, namely, *XXX* and *YY;* two live, namely *XXY* and *XY*. The former is a double yellow female like the parent female, while the other is a male like the father.

Fig. 5. The normal group of chromosomes from the female of D. melanogaster is shown in *a*. The group of chromosomes of a haplo-IV female is shown in *b*. The group of chromosomes of a tripo-IV female is shown in *c*.

a recessive factor in one of its IV chromosomes, eyeless for example, the offspring are expected to be of two kinds as shown in the diagram (fig. 6). Half of them are triplo-IV, and half are normal for these chromosomes. Suppose now one of these F_1 triplo-IV flies is back-crossed to an eyeless fly (from stock), the offspring are in the ratio of five wild type to one eyeless instead of equality as in ordinary cases of heredity where a back-cross is made. An analysis of the kinds of germ cells expected after maturation of the eggs or sperm of a triplo-IV

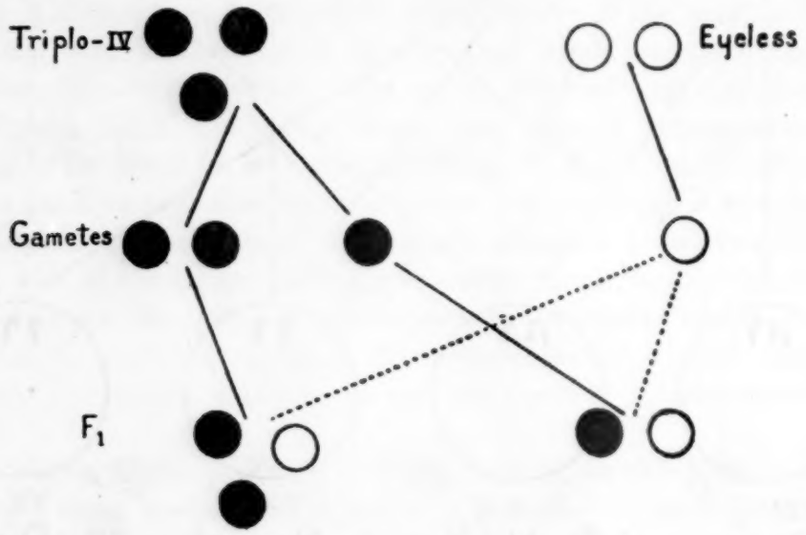


Fig. 6. The result of mating a triplo-IV to an eyeless fly is represented by the chromosomes that are specially involved. The triplo-IV (three black circles), is supposed to carry normal allelomorphs; the other fly, that is eyeless, is supposed to carry the recessive gene for eyeless in the fourth chromosomes (represented here by the open circles). The gametes of these two kinds of flies are represented in the second line, and their recombinations in the third line. Two kinds of individuals result in this first F_1 generation, namely, a wild type that is triplo-IV, and a wild type that is diplo-IV i.e., a normal fly. For a test of the inheritance shown by the former, see figure 7.

individual (fig. 7) explains fully the departure from the Mendelian expectation.

The exceptional genetic behavior shown by the triplo-IV flies based on the theory that they have three IV chromosomes has been confirmed by cytological preparations that demonstrate the presence of three IV chromosomes.

In contrast to the triplo-IV individuals other individuals have been found by Bridges in which there is only one IV chromosome (fig. 5c).

Since only one IV chromosome is present only half of the germ-cells are expected to carry this chromosome, hence if such a fly is bred to a normal fly with two IV chromosomes and homozygous in a recessive IV chromosome factor, eyeless for example, half of the offspring are haplo-IV and these are also eyeless. They come from the egg lacking a IV chromosome, fertilized by a sperm bearing a recessive factor (eyeless) in its IV chromosome. The other half of the offspring are

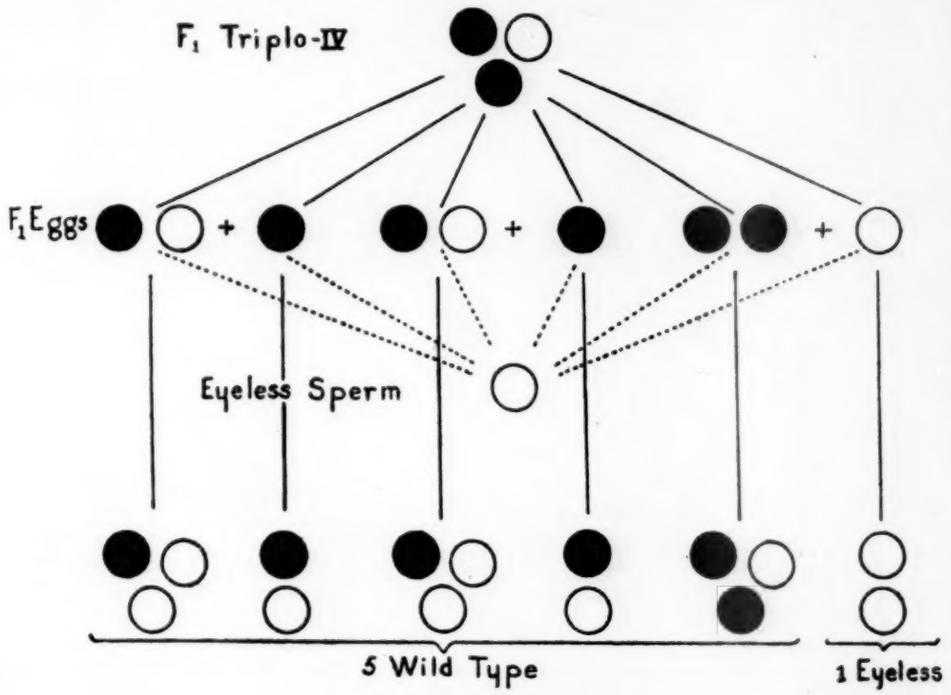


Fig. 7. A back cross of a triplo-IV fly (heterozygous in one chromosome for eyeless; see fig. 6) to an eyeless fly. The three chromosomes of the former are represented at the top, first line; the kind of gametes expected are shown in the second line; the single kind of sperm cell produced by the eyeless fly is represented by the open circle in the middle of the diagram; and in the lower line the six kinds of individuals that result are shown. The expectation is 5 wild type to 1 eyeless.

wild type flies (heterozygous for eyeless). Here then a known recessive Mendelian character appears in half of the first generation offspring. This exceptional result is at once accounted for by the known changes in the number of the chromosomes.

It is probably not a mere coincidence that it is a change in the small IV chromosome that gives these results because its absence is not fatal to the life of the individuals so constituted. No such results

have been obtained for the two large chromosomes, II and III. It would seem, *a priori*, not unlikely that an increase or a decrease in these might so upset the balance, (the effects produced by all the genes) that such an individual would not live. This anticipation has been confirmed by the kinds of offspring produced by still another type of fly in which all of the chromosomes are in threes. These triploids have been found and studied by Bridges both by genetic and by cytological methods. The flies themselves are essentially like the wild type, but a little larger (fig. 8).

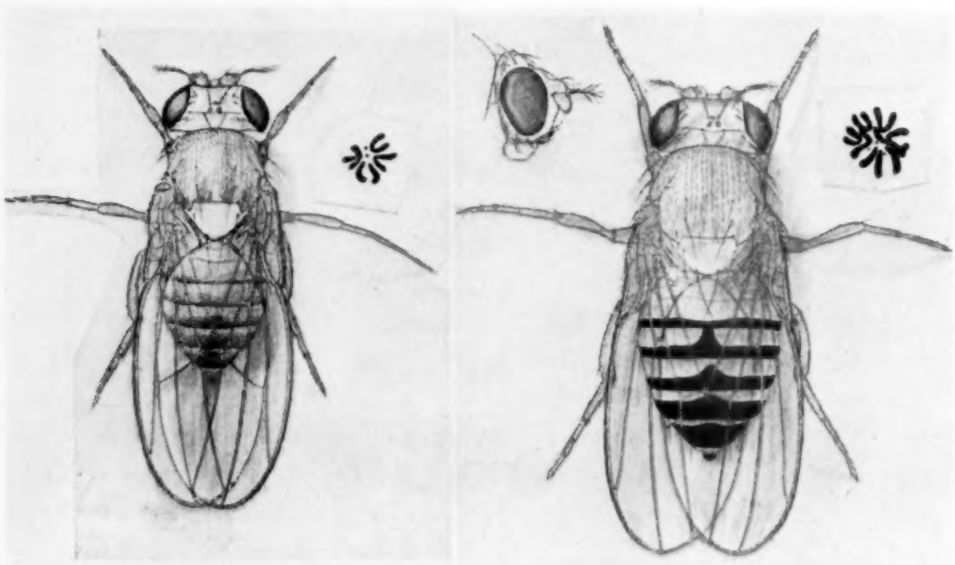


Fig. 8. A normal female (D. melanogaster) to the left and a triploid female to the right. The two chromosome groups are shown above and to the right of the respective females.

When the germ-cells of the triploid pass through the reduction period, each set of three behaves in a non-disjunctional way. The result is that there are many combinations of chromosomes possible. Most of these combinations are not viable (after fertilization), and no flies are found in which chromosome II or chromosome III is in triplicate while the rest are in duplicate (normal). It follows that while all the genes may be in twos or threes (or even fours) and the individual containing them may be perfectly viable, an unbalance in one of the sets, if it is large, causes disaster.

Of course it might well be in particular cases that a very small chromosome might contain factors so essential to the life of the individual

that its loss or even its duplication might be fatal. Nevertheless it seems probable, this being conceded, that, in general, when only a few chromosome pairs are present, a change in one set is more likely to work disastrously than a similar change when a large number of chromosomes are present. However, much remains here for the future to decide.

The relations of the large X chromosomes of Drosophila (and of many other forms) may seem to stand in contradiction to the statement just made, because one of them is absent from one sex, the male, yet that sex is as viable as the other. We can suppose here that the relations have been gradually brought about by compensating effects elsewhere, i.e., in other chromosomes. In fact this situation is actually utilized, so to speak, to produce two extreme types of individuals within the same species, the male and the female. That the sex chromosome arrangement is intimately interrelated with the effects produced by the other chromosomes is known by the occurrence of intersexes amongst the offspring of the triploid.

Physiological effects of the genes. Concerning the ways in which the genes produce their effects genetics has almost nothing to say. There are however certain implications that need to be carefully scrutinized, since it is almost inevitable that speculative views will be advanced concerning the actions of the genes that have little or no foundation in genetics, however probable, or improbable, they may appear from the embryological standpoint.

Mendel used arbitrary symbols (A and a) for the character pairs that he studied. The symbol stood both for the character and for whatever it is in the germ-cells that is there the forerunner of the character. In principle, we use the same scheme today, and since the same symbol stands for both character and gene there is present an opportunity at times for a careless shifting of meaning. In most situations this double meaning of the symbol does no harm, but it has its dangers when, for instance, the variability shown by the character (that may be due to environmental or to modifying genetic factors) is referred to as a property of one gene. Thus, for instance, a rabbit with ears larger than others of its stock might be said to owe its advantage to external factors acting during development or to a different combination of inner factors (genetic) that affect ear length, or to the principal gene affecting ear length being somewhat larger (and therefore more effective) than the average gene. There are well recognized methods by which it can be determined which of these possi-

bilities is responsible for the larger ear of a particular individual. This evidence has shown that results like these are not due to a "larger." gene but either to the environment or to other inner, genetic factors. If the gene were labile, as implied in the last interpretation of variability, the study of heredity would be an extraordinarily complex problem that could be followed only as a mass phenomenon by means of statistical methods. Fortunately there is a large body of information that shows that in heredity we are dealing with individual units of a high order of stability. It is chiefly owing to this that the modern theory of heredity has made rapid progress.

These statements need not be interpreted as dogmatic assertions that the gene is an absolute unchanging unit, that it has no fluctuations, and in this respect comparable to an organic molecule. In the first place, we are not concerned with absolute distinctions. All that we are justified in stating is that by the assumption of stable elements in the germ-material the results we obtain can be explained.

By a stable element we might mean one that maintains a constant size or constitution, or mean only that if it fluctuates quantitatively about a mean, this mean remains the same through countless generations of cell-divisions and generations of individuals. Genetics contributes nothing here, and has in fact no particular interests involved, for either situation would fulfil its requirements.

If our information were more complete, it might be possible from the evidence of crossing-over to discover limiting values for the size of the gene, and find out in this way whether the order of magnitude is such that it comes within the limits of organic molecules. A few tentative estimates of this kind have in fact been made (see Morgan, The Croonian Lecture, 1922), but such attempts, at present, are too uncertain to give the kind of information essential to settle the point under discussion.

How the genes produce their effects through the medium of the cytoplasm of the cell, where differentiation becomes realized, we do not know. It has been suggested that the genes are enzymes. Interesting as are such views from a purely speculative point of view, they have at present little to support them. It is, of course, to be hoped that in time the combined attack on this problem of development both by genetics and by experimental embryology and especially by chemistry may lead to the discovery of the physiological action of the genes, but for the present we must confess ignorance.

There is a rather wide-spread interpretation of Mendelian recessive genes as absences. This interpretation began almost immediately with the recognition of Mendel's paper (1900) and has taken the popular fancy. As a pictorial interpretation, or as a kind of symbolism, one need not quarrel with such a view, for it means nothing more than that small *a* means the absence of a character (A). On the other hand if the symbol is taken in a literal sense it may do mischief if in no other way than making an unwarrented interpretation of the internal factorial scheme of Mendelian inheritance. It may not be out of place to give a little space to this question.

It will be advantageous also to consider along with the view that a recessive character means an absence in the germ-material, another view that has grown up beside the other, namely, that a dominant factor is something added to the original complex of factors. The artificiality of these interpretations is obvious. They owe their origin to the common observation that recessives are often due to loss—loss of color, for example—while some dominants appear as additions, enlargements of the original characters. But it is illogical to conclude that there must be corresponding losses or additions of genes in the germ-material, for the results may be due to any kind of change in the germ material that affects the end products of development in such a way that they may be either losses or gains. It must, of course, be granted that a loss of a gene might cause a loss of a character, and that the addition of a gene might cause the increase of a character, yet there is nothing at all in the genetic evidence that supports such an inference and there is something at least that indicates its absurdity. For example, more than 20 dominants are known in Drosophila. The genetic evidence indicates that a gene has changed (as seen in its effects) but the number of genes remains the same.

Furthermore, the arbitrary nature of the presence and absence interpretation becomes at once manifest when we recall that the distinction between a dominant and a recessive character is by no means as sharp as implied. On the contrary dominance and recessiveness represent rather the extreme end of a continuous series, rather than alternative conditions. A great many characters are neither dominant nor recessive, but intermediate in the heterozygote. Nevertheless their segregation is as clear as when the character pairs contrasted are strictly dominant or recessive. It would not be a gross exaggeration to say that there is no such thing as complete dominance or recessiveness, but in all heterozygotes the influence of both genes may be pres-

ent, although sometimes difficult to detect, except under exceptional conditions. It follows from this that there are no sufficient grounds for the presence and absence hypothesis as literal interpretation. It is needless to go into further details, but it may be pointed out that there are many cases known in which an addition to a common character—as in some melanic types—is produced by a recessive gene, and where a loss of a character—as in brachydactyly—is produced by a dominant gene.

When we consider those cases that are known to result from the absence of a group of genes, or by the addition of a group of genes—as in the haplo- or triplo-IV chromosomes of Drosophila—there is nothing in the kind of character changes to suggest that one causes losses, the other additions. In fact, the evidence points exactly to the opposite conclusions, for, while the absence of a IV may make the wing longer it also makes it narrower and the presence of an additional IV makes the wing shorter but wider, etc.

Cytoplasmic inheritance. There are a few instances known in which self-perpetuating bodies that lie in the cytoplasm produce effects that are inherited. The chlorophyll bodies in plants furnish the best example. Since these, so far as is known, are transmitted only through the cytoplasm of the egg, their inheritance is said to be cytoplasmic. If this form of inheritance were general it would have been frequently met with, but aside from chlorophyll inheritance there are no certain cases of inheritance through the cytoplasm, while practically all the genetic evidence can be interpreted in terms of chromosome inheritance.

The fact that modern genetics has dealt so exclusively with chromosomal inheritance to the exclusion of the cytoplasm has aroused some antagonism from a few embryologists and physiologists, but for somewhat different reasons. The embryologist observes the developmental changes taking place in the cytoplasm in the presence of the entire chromosome complex. He is therefore strongly impressed with the view that the cytoplasm of the cell is the active agent in development. In this he is, of course, entirely right, because his evidence is only concerned with what he can see and not with what takes place behind the scenes. The genetic evidence is the only means we have at present to show that the chromosomes influence what takes place in the cytoplasm.

The physiologist is concerned with processes going on in the cell, but has no means of finding out at present how far the chromosomes may or may not have affected the process. He is, however, as a rule

sufficiently broad-minded to concede at least that any change in the complex of factors present in a cell may affect the end result. Genetics supplies this evidence; it is not concerned with the processes that take place either before or after the postulated change—at least only secondarily interested. Whether or not genetics may in time develop new methods that make possible an approach to some of the problems that at present belong to the field of experimental embryology and physiology remains to be seen, but there is no conflict at present between these studies and no grounds for prejudice. The small amount of prejudice that exists is due to a misconception of the limitations that the geneticist places on his own work.

The question remains nevertheless as to how discrete particles in the chromosomes can produce effects on the cytoplasm of the cell of such a sort as to determine the developmental process and through it the later physiology of the cell. Whether the genes are active all the time or only under specific conditions we do not know, but at least it may be granted that there is no evidence to indicate that all of them are not always at work. How they work we do not know. Several suggestions have been made that are purely speculative or fictitious. The only important contribution that genetics has made in this field may be illustrated by an example. If a red-eyed female Drosophila that has a red-eye-producing gene and a white-eye-producing gene (i.e., if she is heterozygous for white) is mated to a pure white male, half the daughters are red-eyed, half white-eyed. The latter are indistinguishable from white-eyed females, all of whose parents for generations have been white-eyed. This means that the presence of a red-producing gene in the egg of the mother (and in the generations of oöcytes that by division have produced the egg) has had not the slightest effect on the character of the white eye of those of her daughters that have two white genes. This evidence is explicit in showing that even if the presence of the red-producing gene in the egg has affected the cytoplasm of the egg up to the time of maturation, that effect is done away with by the introduction of a white-producing gene at the beginning of embryonic development. It is this kind of evidence from genetics that shows the minor rôle in inheritance played by the cytoplasm. It is needless to point out, perhaps, that the evidence in no sense indicates that the cytoplasm may not be as important for the development as are the chromosomes—only it is under their influence.

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The main purpose of PHYSIOLOGICAL REVIEWS is to furnish a means whereby those interested in the physiological sciences may keep in touch with contemporary research. The literature, as every worker knows, is so extensive and scattered that even the specialist may fail to maintain contact with the advance along different lines of his subject. The obvious method of meeting such a situation is to provide articles from time to time in which the more recent literature is compared and summarized. The abstract journals render valuable assistance by condensing and classifying the literature of individual papers, but their function does not extend to a comparative analysis of results and methods. Publications such as the Ergebnisse der Physiologie, the Harvey Lectures, etc., that attempt this latter task, have been so helpful as to encourage the belief that a further enlargement of such agencies will be welcomed by all workers. It is proposed, therefore, to establish a journal in which there will be published a series of short but comprehensive articles dealing with the recent literature in Physiology, using this term in a broad sense to include Bio-chemistry, Biophysics, Experimental Pharmacology and Experimental Pathology.

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